PHOSPHORUS CYCLING IN ORGANIC SYSTEMS

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By

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ABSTRACT

Soil phosphorus (P) is often unavailable in SK soils due to immobilization by microbial biomass and complexation with cations. The prohibition of synthetic fertilizer use in organic systems means farmers rely on crop rotation or approved inputs to supply P for crops. Legumes in crop rotation add P to soil through decomposition and deposition, and approved fertilizers such as bone meal (BM), rock phosphate (RP), and composted manure add P to soil through dissolution.

Arbuscular mycorrhizal fungi (AMF) improve crop access to soil P. The fungi colonize roots of host crops, allowing roots to reach immobile pockets of soil P. Colonization by AMF is usually decreased or delayed following partial fallow periods, non-host plants, and the addition of soluble P fertilizers.

This thesis consists of two studies. For the first, the effects of crop rotation were tested on AMF colonization and soil P dynamics. Colonization by AMF of mycorrhizal crops was examined following a non-mycorrhizal crop, a partial fallow period, and mycorrhizal crops. All crops were colonized evenly (63-70%) at flowering despite non-mycorrhizal and partial fallow periods, and the sequence most depleted in soil N (wheat-barley) had the lowest colonization in August (36%).

The second study evaluated soil P and plant N and P after applications of BM, hydroxyapatite (HAP), and sheep manure compost. Compost application increased plant P uptake compared to the control (1.26 vs. 0.71 mg pot^{-1}), while applications of BM and HAP alone did not. Compost application did not affect AMF colonization of wheat (*Triticum aestivum* L.).

Overall this research highlights the importance of legumes and composted manure use in organic systems. Legume use in crop rotation simultaneously increased soil P deposition and may have preserved AMF communities despite fallow periods and non-host crops in rotation. Conditions normally affecting AMF colonization in conventional systems did not apply. The use of composted manure in the greenhouse study resulted in the greatest P uptake and concentrations in wheat. Thus the use of legumes and composted manure may increase P availability to crops directly and indirectly: directly through soil P deposition and perhaps indirectly through the preservation of AMF communities.

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DEDICATION

I dedicate this thesis to all of my friends in Saskatoon and my family in the US. This was a long journey, and would have been impossible without their emotional support.

TABLE OF CONTENTS

page

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AAFC	Agriculture and Agri-Food Canada
ACS	Alternative Cropping Study
ALPase	Alkaline phosphatase
AMF	Arbuscular mycorrhizal fungi
ANOVA	Analysis of variance
Ap	Cultivated A horizon
B	Barley
BM	Bone meal
C_{mic}	Microbial biomass carbon
CNC	Critical nutrient concentration
CNR	Critical nutrient range
Ct	Control
cv	Cultivar
dia	Diameter
DAE	Days after emergence
DAG	Diverse annual grain
DAP	Diverse annual perennial
DNA	Deoxyribonucleic acid
FC	Field capacity
FID	Flame ionization detector
GMO	Genetically modified organism
HAP	Hydroxyapatite
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulfuric acid
$H_2PO_4^-$	Orthophosphate ion
HPO_4^{2-}	Orthophosphate ion
k_{EP}	Efficiency of microbial biomass phosphorus extraction
LGrM	Lentil green manure
LSD	Least significant difference
LOW	Low diversity
M	Mustard
MIDI	Microbial ID Inc
MS	Mass of soil
MUB	Modified universal buffer
N_{mic}	Microbial biomass nitrogen
OD	Optical density
P_F	Phosphorus in fumigated samples
P_i	Inorganic phosphorus
P_{mic}	Microbial biomass phosphorus
P_o	Organic phosphorus
P_{UF}	Phosphorus in unfumigated samples
PLFA	Phospholipid fatty acid analysis
PNP	*p*-nitrophenyl phosphate
PP	Pea

PS	Pre-seeding
R	Percentage recovery of inorganic phosphorus
RP	Rock phosphate
SMB	Soil microbial biomass
SMC	Soil moisture content
ShMC	Sheep manure compost
SOM	Soil organic matter
SpD	Spore density
SPE	Solid phase extraction
SPSS	Statistical Package for the Social Sciences
TSE	Transmissive spongiform encephalopathies
VS	Volume of solution in extracted soil (Microbial biomass phosphorus)
W	Wheat

1. INTRODUCTION

Due to the highly calcareous nature of SK soils, phosphorus (P) availability is generally very low. The immobilization of soil P results from the formation of insoluble compounds with calcium and immobilization of P by the soil microbial biomass (SMB) (Oberson et al., 1996; Malhi et al., 2002). Phosphorus is one of the major nutrients required for plant growth, and its nutrition is especially important in the early season of most crops (Tisdale and Nelson, 1975). Without sufficient P concentrations, crops may show stunted growth, slowed maturity, and impaired growth of reproductive organs (Tisdale and Nelson, 1975).

The unavailability of soil P presents challenges for organic farmers throughout SK. Unlike conventional farming systems, organic certification prohibits the use of synthetic fertilizers (Canadian General Standards Board, 2011). This means that to supply the P needed for plant growth and development, organic farmers must rely on soil amendments approved by certifying agencies (Canadian General Standards Board, 2011) or nutrient inputs from crop rotation. Unlike most organic farming systems, the use of manure for P supply is rare on organic farms in SK, due to small average size of herds and large average size of farms (Knight et al., 2010a).

Crop rotation is a vital component to any organic system (Stockdale et al., 2001; Akter et al., 2004). It is designed to ensure that crops with heavy nutrient demands will follow those that build soil fertility (Stockdale et al., 2001). When properly implemented, crop rotations help with disease, nutrient, weed, and pest management, as well as maintain soil fertility over time (Stockdale et al., 2001). Generally, crop rotations on organic farms in SK include cereals,

oilseeds, and legume green manures (Knight et al., 2010b). Cereals (i.e. wheat, barley) and oilseeds (i.e. mustard) are heavy nutrient users, while legumes (i.e. lentil, alfalfa) build soil N through biological nitrogen (N_2) fixation (MacLeod, 1999) and release N and P into soil following their decomposition when used as green manures (Hargrove, 1986; Sharma and Mittra, 1988; Biederbeck et al., 1998).

Arbuscular mycorrhizal fungi (AMF) are well-established in their role of assisting in plant P uptake (Bolan, 1991; Smith and Read, 1997) and colonize approximately 80% of plants on earth (Zhu et al, 2007). Colonization of crop roots by AMF increases root surface area through extensive hyphal networks, allowing crops to explore greater areas of the soil profile and access soil P that is otherwise unavailable (Smith and Read, 1997). The degree of crop colonization by AMF is generally greater in organic than conventional systems (Mäder et al., 2000; Oehl et al., 2003; Entz et al., 2004), which may be due to the decrease of colonization following additions of soluble P fertilizers or other P amendment sources (Asimi et al., 1980; de Miranda et al., 1989; Hinsinger, 2001). It has also been observed that AMF colonization is delayed after a non-mycorrhizal crop or fallow period in rotation, due to the absence of hosts and disruption of hyphal networks (Gavito and Miller, 1998; Karasawa et al., 2002; Bedini et al., 2007).

There are certain P amendments that are approved for use on organic farms. Composted manure is a valuable source of P, due to the preservation of P during the composting process (Adler and Sikora, 2003). Rock phosphate (RP) and bone meal (BM) are other soil P amendments approved in organic farming, although the availability of P from each is usually greater in soils with pH<6 (Bekele and Hofner, 1993; Surendra et al., 1993). This means that RP

has shown mixed results in SK soils, as soil pH is generally >6 due to calcium carbonate in the parent material (Malhi et al., 2002).

1.1 Organization of the Thesis

The overall purpose of this thesis was to assess the problem of soil inorganic P deficiency on organic farms in SK, and to research possible solutions to increase soil inorganic P for crop uptake. The research was based around one main question: What are practical ways for organic farmers in SK to address shortages of inorganic soil P? Crop symbiosis with AMF was identified as an important aspect in these systems due to enhanced crop uptake of P. The P status of soil (microbial biomass P and alkaline phosphatase) and crops (crop P concentration and uptake) were also necessary to gain a complete picture of the soil-plant system. Crop N concentrations and uptake were also analyzed in both experiments because concentrations of N in plant tissue are closely related to availability of P (Tisdale and Nelson, 1975).

This thesis is divided into two studies. The first study analyzed soil P dynamics and crop colonization by AMF in a long-term organic system without any inputs, and is done entirely in the field aside from a bioassay in the greenhouse. Four cropping sequences were analyzed. One cropping sequence contained a partial fallow period (lentil green manure), one a non-mycorrhizal crop (mustard), and the remaining two sequences contained uninterrupted mycorrhizal colonization. Objectives involved (i) quantifying alkaline phosphatase (ALPase) activity, microbial biomass P (P_{mic}), and N and P uptake from each cropping sequence, (ii) determining if the number of AMF spores, AMF DNA, and AMF colonization of mycorrhizal crops decreased after non-mycorrhizal crops, and (iii) determining if the non-mycorrhizal crop and partial fallow period delayed AMF colonization of mycorrhizal crops following them.

The second study was conducted entirely in the greenhouse, and its purpose was to examine the impacts of BM, hydroxyapatite (the main component of RP), and sheep manure

compost (ShMC) applications on AMF colonization of wheat, ALPase activity, P_{mic}, and plant N and P nutrition.

2. LITERATURE REVIEW

2.1 Organic Farming

Over the last few decades, many farmers have turned to organic rather than conventional farming due to environmental and health concerns. The organic industry has steadily grown over this time period, and is described as "the most dynamic and rapidly growing sector of the global food industry" (Agriculture and Agri-Food Canada, 2011).

In Canada, organic production and consumption have increased since the 1980's (Agriculture and Agri-Food Canada, 2011), and between 1992 and 2010, the number of Canadian organic farms grew from approximately 1000 to 3500 (Canadian Organic Growers, 2011). Quebec has the greatest number of organic producers (1015) followed by Saskatchewan (963) (Canadian Organic Growers, 2011). The focus of organic production varies throughout the country. For example, the majority of Canadian organic fruit, vegetable, and greenhouse production is in British Columbia, and the greatest production of organic maple syrup is in Quebec (Kendrick, 2008). In the Prairie Provinces, field crops are the main component of organic acreage (Macey, 2010).

Organic farming is an important industry in the Canadian Prairies (Macey, 2010). Farming in this region presents many challenges, however. The Canadian Prairies have very cold winters, short growing seasons, and frequent drought conditions (Knight et al., 2010a). Furthermore, the use of manure, a valuable source of organic fertilizer, is rendered largely impractical by the massive average size of farms (Knight et al., 2010a; Canadian Organic

Growers, 2011). The number of cattle on organic farms in the Canadian Prairies is rarely large enough to supply manure for the entire farm (Knight et al., 2010a).

All organic farms in Canada must comply with the Canadian Organic Standards outlined by the Organic Product Regulations (Canadian General Standards Board, 2011). Organic food must be certified by the appropriate certifying bodies, which are accredited by conformity verification bodies accepted by the Canadian Food Inspection Agency (Canadian Organic Growers, 2011). Canadian organic principles and management standards prohibit the use of any genetically modified organisms (GMO), cloned farm animals, ionizing radiation on food products, antibiotics, food additives, nanotechnology products or processes, synthetic food aids or additives, and synthetic growth regulators (Canadian General Standards Board, 2011). Furthermore the use of pesticides, inorganic fertilizers, sewage sludge as soil amendment, wood preservatives, or fertilizers and compost material containing any of the prohibited substances is not allowed (Canadian General Standards Board, 2011).

The organic certification process requires that none of the prohibited materials have been used on farmland for at least 36 months, and that the entire farming operation has transitioned to organic throughout this time period (Canadian General Standards Board, 2011). Farmers must provide receipts and documentation as proof. If contact with prohibited substances is possible from neighboring fields, farmers must build physical barriers (e.g. windbreaks, permanent hedgerows, and buffer zones). Any crops grown within buffer zones or windbreaks are not considered organic (Canadian General Standards Board, 2011).

The main focus of organic farming is on the system as a complete, long-term solution for long-term health (Stockdale et al., 2001). Central to the philosophy is the interdependency of all components (Köpke, 1995), and emphasis is placed on the preservation of water, organisms, air,

and soil quality. Some studies have shown greater soil nutrient cycling and soil biological activity in organic than conventionally farmed fields (Drinkwater et al., 1995; Carpenter-Boggs et al., 2000; Fliessbach and Mäder, 2000; Bulluck et al., 2002). In practice, however, the soil fertility status of organic farms is varied. Without the use of inorganic fertilizers, the availability of soil nutrients for plants is often limited compared to conventionally-managed soils. For example, soil nitrogen (N) is commonly lacking in organic systems (Watson et al., 2002b), as is soil phosphorus (P) (Entz et al., 2001; Malhi et al., 2002; Knight and Shirtliffe, 2003). Both of these nutrients limit plant growth when not present in sufficient quantities.

Maintaining biodiversity is also a vital part of the organic philosophy (Tamm, 2001). This includes biodiversity in crop rotation and in weed and organism populations. Greater diversity of weed species in organic than conventional farms is often observed, as well as greater diversity of microorganisms (Mäder et al., 2002; Chen et al., 2005). Weeds are viewed as habitat for beneficial insects (Turner et al., 2007), and the diversity of beneficial microorganisms is necessary for soil nutrient cycling (Mäder et al., 2002).

The presence of weed populations presents many challenges for organic farmers. Often organic producers perceive weeds as a greater threat to their farms than the unavailability of nutrients in soil (Knight et al., 2010a). Competition with weed populations for nutrient and water resources can reduce grain yields in any system, but the greater weed diversity and abundance on organic farms presents a particular challenge (Mason and Spaner, 2006). In the Canadian Prairies, organic grain yields are lower than in many other areas where similar studies were conducted (Snyder and Spaner, 2010). On average, fourteen farms surveyed in MB, SK, and North Dakota had yields of hard red spring wheat (*Tritucum aestivum* L.), oats (*Avena sativa* L.), and barley (*Hordeum vulgare* L.) that were 77%, 73%, and 74%, respectively, of those in long-

term conventional systems (Entz et al., 2001). This was partially attributed to the presence of weed species in the organic farms.

Another core principle of organic farming is sustainability. Emphasis is placed on using locally-sourced and recycled materials whenever possible, as well as maintaining system diversity and soil fertility. Sustainability on organic farms refers not only to the farming practice itself, but also to its economic and social sustainability. Due to the higher prices of organic products on the market and the lower costs of pest control and inputs on-site, organic farms can be more profitable than conventional farms (Stockdale et al., 2001; Pacini et al., 2003). Overall, the sustainability of organic systems may be greater than in conventional systems (Pacini et al., 2003).

Supporters of organic agriculture claim that organic systems have reduced greenhouse gas emissions and improved energy use efficiency compared to conventional systems (Lynch, 2009), but this is dependent on management practices. A study in MB over twelve years compared the use of grain-based and integrated crop rotations of conventional and organic farms (Hoeppner et al., 2006). This study concluded that the organically-managed, integrated cropping systems used energy the most efficiently. However, they were also the most deficient in soil P. It is possible that the energy requirements for long-term soil P management would cancel out any short-term energy efficiency.

Further studies show that the integration of certain organic management practices with conventional systems may offset energy costs over the long-term (Pimentel et al., 2005; Ammann, 2008 and 2009; Snyder and Spaner, 2010). One study found that certain organic techniques used in a conventional system offset many of the negative environmental impacts (Pimentel et al., 2005). In particular, using natural biodiversity could reduce the use of N

fertilizers, herbicides, insecticides, and fungicides. This indirectly reduces environmental impact, cost to the farmer, and the use of fossil fuels for fertilizer, herbicide, insecticide, and fungicide production (Pimentel et al., 2005). It is therefore possible that conventional systems can be as energy-efficient as organic ones, and that energy use and environmental sustainability are heavily dependent on management practices (Snyder and Spaner, 2010). The energy-use efficiency of any organic farm therefore depends heavily upon the choices of the producer.

Organic systems are often criticized because of their reliance on tillage. Conventional tillage enhances microbial activity and increases decomposition rates due to enhanced availability of oxygen and closer contact of residue and soil, which translates to greater mineralization and nutrient availability to plants (Lupwayi and Burr, 2010). Despite the benefits to plants, however, the practice renders the soil bare and prone to erosion. Loss of topsoil can result in the system's dependence on cultivation to render nutrients plant-available (Gliessman, 2007).

2.2 Soil Fertility in Organic Systems

Soil fertility is a main determinant of food production, and soil degradation is one of the greatest threats to food security (Stockdale et al., 1992; Reganold et al., 1993; Drinkwater et al., 1998; Pretty et al., 2000; Stockdale et al., 2001; Bloem et al., 2005). Fertile soil promotes system sustainability through plant-available nutrients, active and diverse microbial communities, and healthy soil structure suitable for root growth and drainage. Generally, nutrient levels on organic farms are considered to be lower than their conventionally-managed counterparts, however nutrient status is closely related to the organic farmer's choice of inputs and the length of time the soil is under organic management (Watson et al., 2002b; Gosling and Shepherd, 2005).

Soil organic matter (SOM) is a vital aspect of soil fertility. It is important for storing nutrients, preventing soil erosion, accelerating decomposition, and improving soil structure

(Malhi et al., 2010). According to the Canadian General Standards Board (2011), organic practices must maintain or increase humus levels to preserve soil fertility over time. Humus is the portion of SOM most resistant to decomposition (Tisdale and Nelson, 1975). Organic farmers cannot rely on the use of inorganic fertilizers, so the maintenance of this nutrient pool is vital. The main methods of increasing SOM in organic systems involve additions of organic matter (usually manure) and live plant material, although the success of these methods relies on the availability of manure and the type of crop residue (Knight et al., 2010b). The large average farm size in SK and absence of livestock limits the use of manure. Therefore, organic farmers in SK rely mainly on the use of crop residues for SOM build-up (Knight et al., 2010b).

When applying plant material to sustain SOM levels, it is important to consider the rate at which it decomposes. Decomposition and activity of microbial populations is affected by environmental factors such as soil moisture and temperature (Jenkinson and Ladd, 1981). Microbial decomposition increases in warm (>4 °C), moist conditions and neutral pH (7), although water-logged and poorly aerated soils will inhibit it (Macduff and White, 1985; Pilbeam et al., 1993; Grant, 1994). Another factor influencing the decomposition of SOM is the quality of the residue. Mineralization and immobilization of crop residues are affected by the C:N and C:P of the residue (Tisdale and Nelson, 1975). Mineralization refers to the conversion of N and P from plant-unavailable (organic) to plant-available (inorganic) forms, while immobilization is the conversion of inorganic N and P to organic N and P, rendering it unavailable for plants (Tisdale and Nelson, 1975). Mineralization represents microbial decomposition, and immobilization represents microbial assimilation (Tisdale and Nelson, 1975). Crop residues with narrow C:N ratios (<20) are rich in N. Their application to soil results in net mineralization as excess N is released in the form of plant-available N through microbial decomposition. These

residues may decompose too quickly to add significant amounts of organic matter to SOM (Lupwayi et al., 2006a). Crop residues with wide C:N ratios (>30) induce net immobilization initially due to microbial assimilation of inorganic N, which is eventually released back into the system as microbes die (Tisdale and Nelson, 1975). Crop residues with wide (C:N>30) ratios are more likely to result in build-up of SOM than crop residues that decompose quickly, as N is released over time (Lupwayi et al., 2006b). When C:N falls between 20 and 30, both processes occur without a net accumulation of either. The mineralization and immobilization of P behaves similarly. Generally, when C:P<200, net mineralization occurs, and when C:P>300, net immobilization occurs (Tisdale and Nelson, 1975). Between these ratios each process occurs without a net accumulation of one over the other (Tisdale and Nelson, 1975). Mineralization and immobilization of P and N in SOM are also related to C:N:P ratios. While the ratio varies across soil types, SOM has a general C:N:P ratio of 100:10:1 (Tisdale and Nelson, 1975). When N is limiting and P is available, this induces mineralization of P that is too rapid for SOM accumulation (Nziguheba et al., 2000; Kwabiah et al., 2003a). The reverse occurs when P is limiting.

Some organic systems have higher SOM levels than conventional systems (Scullion et al., 2002; Shannon et al., 2002; Marriott and Wander, 2006; Teasdale et al., 2007; Tuomisto et al., 2012), while others show no difference in SOM levels between conventional and organic systems (Campbell et al., 1991; Wander et al., 2007). The variations in tillage of these systems may be factor, as it is widely known that tillage practices can reduce SOM by accelerating its decomposition (Doran 1980).

In SK, SOM levels are often slightly lower in organically- than conventionally-managed soils (Knight et al., 2010b). This may be a combination of lack of manure inputs, reduced tillage

in conventional systems compared to organic systems, and the quality of crop residues returned to the soil. A six-year study conducted by Biederbeck et al. (1998) in Swift Current, SK compared the SOM inputs from Tangier flatpea (*Lathyrus tingitanus* L.), chickling vetch (*Lathyrus sativus* L.), feedpea (*Pisum sativum* L.), and black lentil (*Lens culinaris* Medikus) in rotation with wheat versus a continuous wheat rotation and a wheat-fallow rotation. They found that the flatpea, chickling vetch, feedpea, and black lentil returned more SOM than the fallow-wheat rotation, but similar amounts to the continuous wheat rotation. Crops with the greatest N mineralization added the least to SOM pools, and vice versa. Again, crop residue contributions to SOM are dependent on the quality of the plant material.

2.3 Phosphorus in Organic Systems

Phosphorus (P) in soil originates from rocks in the parent material, largely from insoluble apatite components (Tisdale and Nelson, 1975). While plant-available N is partially fixed into soil by rhizobium, microorganisms that exist symbiotically with roots of certain plants, and other free-living N-fixers in soil, P cycles through decaying organisms that deposit a range of P compounds in the soil (Walker and Adams, 1958). Phosphorus diffuses very slowly through the soil profile (Nye and Tinker, 1977). Because this nutrient only moves over short distances, crops with dense and extensive root systems have easier access to soil P than crops with coarse and shallow root systems (Tisdale and Nelson, 1975).

Adequate concentrations of P in plants are important for root growth, plant maturity, and development of reproductive organs (Tisdale and Nelson, 1975). Most plant P uptake occurs in the early stages of growth, which means that plant P nutrition early in the growing season is vital (Tisdale and Nelson, 1975; Malhi et al., 2006). The nutrient deficiency symptoms of P are difficult to identify, as P is very mobile in plants, and moves from old to young tissue once a

deficiency occurs. The most common symptoms of P nutrient deficiency are stunted growth and slowly maturing plants (Tisdale and Nelson, 1975).

There are two main pools of P in soil. The largest pool, which is unavailable to plants, is organic P (P_o). Organic P comprises 5-90% of total P in soils (Cosgrove, 1967). Generally, higher levels of soil P_o are found in soils with higher clay content. This is due to the sorption of phosphate compounds to soil colloids, which dominate clay and silt fractions (Williams and Saunders, 1956).

For soil P_o to become plant available, it must first mineralize to inorganic soil P (P_i). The mineralization of P_o is related to soil C:P and C:N ratios, and indirectly related to C mineralization processes (Joner and Johnasen, 2000). A ratio of C:P>300 will induce net immobilization and C:P<200 will induce net mineralization (Tisdale and Nelson, 1975). Mineralization of P_o also relates to the ratio of N:P, as soil inorganic N deficiencies will result in build-up and mineralization of P_o (Kwabiah et al., 2003a).

Plants are able to take up soil P_i in two forms of orthophosphate ions ($H_2PO_4^-$, HPO_4^{-2}), and the availability of these ions to plants is dependent upon soil pH and the solubility of P-complexes in the soil profile (Tisdale and Nelson, 1975). The $H_2PO_4^-$ ion dominates at lower pH, followed by HPO_4^{-2} ions as pH increases. In solutions of approximately pH 6.5, equal amounts of $H_2PO_4^-$ and HPO_4^{-2} ions are found. However, it is possible for orthophosphate ion adsorption and fixation to occur at all pH levels (Tisdale and Nelson, 1975). In acidic soils, $H_2PO_4^-$ ions dominate, and are mostly fixed to iron (Fe), aluminum (Al), and manganese (Mn) cations. In alkaline soils, such as those found on the Canadian Prairies, HPO_4^{-2} ions dominate, and typically precipitate with calcium (Ca^{2+}) salts (Buckman and Brady, 1969). Typically, soil P is relatively

unavailable to crops in organic systems due to low solubility and uneven distribution (Malhi et al., 2002).

The relatively unavailable soil P in SK presents many challenges for organic farmers. Conventional systems rely on the addition of inorganic fertilizers for soil P fertility, as there are no other significant inputs of P other than what is available from the soil parent material (Tisdale and Nelson, 1975). This translates to low plant P_i availability in organic farming systems throughout the province (Government of SK, 2010). A study by Entz et al. (2001) revealed that eight out of nine surveyed organic farms in MB were deficient in plant-available P (>20 kg ha^{-1}). Average levels of soil P (15 kg ha^{-1}) were lower than those found in conventional fields in MB (20 kg ha^{-1}). Furthermore, the lowest levels of available soil P were detected in the sites that were under organic management the longest (70 and 30 years). Malhi et al. (2002) and Knight and Shirtliffe (2003) also found P deficiencies in organically-managed soils in the Prairies. Without the addition of P fertilizers to cropping systems, available soil P is known to deplete over the long-term (Campbell et al., 1993).

2.4 Crop Rotation

Crop rotation refers to three or more crops grown in a defined and repeating sequence (Vereijken, 1997). Many studies have demonstrated the benefits of complex cropping rotations in farming systems, including weed suppression, increased yield, enhanced nutrient availability, and pest and disease control (Stockdale et al., 2001; Akter et al., 2004; Ciftci and Ulker, 2005; Wang et al., 2012).

Crop rotations in organic systems are especially important, as the use of inorganic N and P fertilizers is not allowed (Watson et al., 2002b). Generally, crop rotations are divided into phases that deplete soil N and phases that build it, although soil P is also an important consideration (Tisdale and Nelson, 1975; Cavigelli and Thien, 2003). In order to maintain long-

term soil fertility of N and P, these phases must be in balance with one another (Berry et al., 2003; Watson et al., 2002b). The improper management of N or P can result in loading, run-off, and eventual pollution of waterways, which is among the greatest environmental concerns associated with any farming system (Snyder and Spaner, 2010; Nelson and Janke, 2007).

A variety of crops have the ability to fix N from the atmosphere. For example, sinorhizobium form a symbiotic relationship with the roots of alfalfa and fix N in this manner. As a perennial crop, alfalfa can be grown multiple years, which builds soil N through decomposition of its residue for heavy nutrient users like wheat or barley (Lampkin, 1990; Watson et al., 2002a). Proper use of crop rotation in organic systems is also important for soil P management. One study reported that P uptake by sorghum (*Sorghum bicolor*) was greater following alfalfa (*Medicago sativa*), red clover (*Trifolium pretense*), and sweetclover (*Melilotus officinalis*) than pea (*Pisum sativum* L.) (Cavigelli and Thien, 2003). Uptake of P by crops is therefore influenced by their place within a crop rotation.

Crop rotation is also related to water and nutrient use. The use of soil water by crop root systems is linked to their root depth, and varying crop types will access different areas of the soil (Watson et al., 2002a). For instance, winter wheat and safflower (*Carthamus tinctorius*) access deeper soil resources than pea (Merrill et al., 2002). Using crops effectively in rotation ensures that soil nutrients and water resources are replenished as needed.

2.4.1 Cereals, legumes, and oilseeds

Cereal crops (e.g. barley and wheat) and oilseeds (e.g. mustard, flax) require large amounts of soil nutrients, while legumes (e.g. pea, alfalfa) can meet some of their N needs through fixing N from the atmosphere (Biederbeck et al., 1998; Saskatchewan Ministry of Agriculture, 2013). Fixation of N can offset potential N losses through denitrification, immobilization, or leaching (Bremer and van Kessel, 1992).

In general, organic crop rotations in the Canadian Prairies will include cereals, oilseeds, and legumes. Low yields of cereals and oilseeds in organic systems are often related to the difficulty of soil nutrient management and synchronization of nutrient demand with availability (Stockdale et al., 1992; Lupwayi et al., 2006b). Benefits from including legumes in crop rotation can include soil N build-up from legume crop residue incorporation and decomposition, greater crop yields, weed control, disease prevention, and improved soil fertility overall (Hoyt, 1990; Beckie and Brandt, 1997; Kelner et al., 1997).

Legumes can build soil N and increase yields of subsequent crops through the decomposition of their residue. An eight-year study in southern SK examined yields and the N contribution from lentil, dry bean (*Phaseolus vulgaris* L.), and dry pea crop residues (Miller et al., 2002). The crop residues added 5, 6, and 9 kg ha^{-1} N more soil N than wheat residue at soil depths of 0 to 120 cm, and wheat yields were 21% greater of wheat grown on pulse stubble rather than wheat stubble (Miller et al., 2002). Another study comparing wheat-wheat and lentil-wheat rotations in Swift Current, SK reported higher N content of wheat in the lentil-wheat rotation in 11 of 18 experiment years (Zentner et al., 2001).

Despite the contribution of N from legume residue, the N benefit from legumes does not consistently offset losses of N through leaching, denitrification, or immobilization. Annual legumes in particular may not provide consistent N contributions (Zentner et al., 1996; Brandt, 1999; Przendowek et al., 2004). A field experiment in MB comparing N benefits to consecutive wheat crops found that of field pea, soybean (*Glycine max* L.), chickpea (*Cicer arientinum* L.), and dry bean, field pea showed higher yields of subsequent wheat than all other treatments (Przednowek et al., 2004). Soybean in particular provided little to no N-benefit to following wheat crops from its stubble (Przednowek et al., 2004).

Legumes can also contribute P to soil through their decomposition (Knight and Shirtliffe, 2005). The C:P ratio of many legumes is narrow (<200), allowing decomposition and release of P in a plant-available form (Cavigelli and Thien, 2003). Sequences of non-legumes succeeding legumes are also beneficial for P plant nutrition. One study reported greater plant P-uptake of a non-legume following a legume than two non-legumes in sequence (MacLeod, 1999). This was attributed to increased soil P availability through enhanced root growth of the non-legume (MacLeod, 1999).

Legumes may reduce the yield of following crops due to their high water demands. On the Canadian Prairies, legumes used in organic cropping rotations are typically perennial species. They may deplete soil resources and even reduce yields of following crops in the Prairies (Campbell et al., 1990; Foster, 1990). Annual legumes have shallower root systems, and may be a better option for organic farmers on the Prairies for soil water conservation although they do not always replace all N required for crops (Biederbeck and Bouman, 1994). When selecting legumes for crop rotation, organic farmers in the semiarid Canadian Prairies must consider their water and nutrient usage as well as their potential N contributions.

2.4.2 Green manure

A green manure is any crop that is ploughed down to add SOM, N, or other nutrients through incorporation (Biederbeck et al., 1998). Often, legumes are used as green manures due to their N-fixing capabilities. Legume green manures are a potential source of renewable and on-farm soil N (Hargrove, 1986; Sharma and Mittra, 1988).

Green manures may add to the SOM pool, although their contributions of organic matter vary. The build-up of SOM from green manures is related to their C:N ratio as well as the time of incorporation. Many young legumes have narrow C:N (<20) ratios, which means that when incorporated into soil they decompose quickly without contributing to SOM pools (Foster, 1990;

Biederbeck et al., 1998). However late incorporation of green manures can result in substantial losses of N through leaching (Foster, 1990). A three-year study in Saskatoon, SK found that early incorporation of the legume green manure sweetclover showed the least amount of N lost through leaching and the highest N availability of any treatment (Foster, 1990). While not measured, they speculated that older plant materials are more resistant to mineralization, and their incorporation could lead to low levels of N availability in spring (Foster, 1990). When using green manures in crop rotation, organic farmers must consider N additions to SOM and potential leaching losses over the long-term versus plant-available N in the short-term.

Green manures can also release P into soil through their decomposition, although the C:P ratio is not always an accurate predictor of P mineralization from these sources. One greenhouse study examining P uptake by sorghum following green manure and other perennial crops found the lowest P uptake following lupin (*Lupinis albus*), the legume green manure with the narrowest (<200) C:P ratio (Cavigelli and Thien, 2003). In fact, P-uptake by sorghum was greatest in the non-green manure crops with C:P~1000 (Cavigelli and Thien, 2003). This indicates that legume green manures are not always a reliable contributor of plant-available P, and that mechanisms governing P mineralization are more complex than those governing N mineralization.

Green manures can be used in place of summer fallow, in which a field is left unplanted over a growing season for improved water storage and extended decomposition of plant residues for increased soil nutrient storage (Greb et al., 1974). However, their water demands mean they are not automatically a better alternative (Campbell et al., 1990; Foster, 1990). As with N availability, the time of incorporation is important for efficient water usage. Early incorporation of sweetclover showed the greatest succeeding wheat yields of any treatment, attributed to increased water availability (Foster, 1990). A 7-year study conducted in Swift Current, SK found

that a wheat-on-wheat rotation recharged 68% of the soil water compared to the fallow-wheat rotation, while the green manure-wheat rotation recharged soil water up to 81% of the fallow treatment (Biederbeck and Bouman, 1994). They concluded that a combination of lentil green manure and fallow preceding wheat may be the best option for water conservation.

The use of non-legumes for green manures is less common and rarely researched within the Canadian Prairies (Knight et al., 2010b).

2.4.3 Critical nutrient concentrations and critical nutrient ranges of crops

When evaluating crop rotations, it is important for farmers and scientists to have a reference for crop nutrient status. The nutrient requirements of crops vary according to nutrient, crop type, the part of the crop sampled (e.g. leaves, stem, seed) and the growth stage of the crop (Tisdale and Nelson, 1975). The critical nutrient concentration (CNC) of a crop refers to optimum crop nutrient concentrations necessary for maximum crop yield and quality, and is an important tool to diagnose crops for nutrient deficiencies (Tisdale and Nelson, 1975). A particular CNC for crops gives a specific answer regarding the status of a specific nutrient, and depends on the type of crop, the nutrient, the time of sampling, the age of the crop, and the part of the crop that is sampled (Tisdale and Nelson, 1975).

However, the use of a single concentration is limiting and not practical, as there are ranges in which nutrients are considered sufficient. Therefore the use of a critical nutrient range (CNR) is more useful (Dow and Roberts, 1981). Below a crop's CNR, nutrient concentrations are considered deficient, within it, they are sufficient, and above it, they are in excess (Dow and Roberts, 1981). This range is usually expressed as a percentage. For example, the CNR for P concentration in pea tissue at flowering is 0.39% to 0.67% (Plancquaert, 1989). Phosphorus concentrations of pea that fall within this range at flowering are at the optimum concentration in the plant tissue for crop yield and quality (Dow and Roberts, 1981). Although these concepts are

generally used to estimate crop fertilizer additions in conventional farming, they identify crop nutrient deficiencies and are useful in organic systems as well.

2.5 Mechanisms for Increasing Plant-Available Phosphorus

Arbuscular mycorrhizal fungi (AMF) are endophytic fungi that form a symbiotic relationship with 80% of terrestrial plants (Zhu et al., 2007). In exchange for a steady supply of C from the host plant, AMF transfers many benefits to its host, including increased soil stability through hyphal growth (Miller and Jastrow, 2000), increased nutrient uptake (Bolan, 1991; Smith and Read, 1997), and increased resistance to disease and drought (Ryan and Tibbett, 2008). It has also been observed that more AMF are found in non-acidic soils (Zhu et al., 2007), such as many of the calcareous soils farmed organically in SK (Malhi et al., 2002).

Among the observed benefits to host plants from AMF infection, the most widespread is increased P uptake (George et al., 1992). Infection by AMF extends plant roots, allowing them to access previously undisturbed regions of the soil profile and immobile soil P (Brady, 1990; George et al., 1992). The nutrient exchange of C from the host plant and P from the fungi occurs between AMF hyphae and plant root cells of the host. Arbuscular mycorrhizal fungi are entirely dependent upon the host plant for C supply (Paul and Clark, 1989), and the intensity of AMF infection often relies upon the root structure of the host plant (Plenchette et al., 1983). Generally, plants with finer and more extensive root systems are less dependent on P-nutrition from AMF colonization, and the opposite is true for coarse roots (Plenchette et al., 1983; Paul and Clark, 1989). For example, pea, with its coarse root system, is usually far more dependent on AMF than wheat, which has very fine and extensive roots (Smith and Read, 1997).

Agricultural management practices affect AMF communities. Monocultures can decrease AMF spore abundance due to low host diversity (An et al., 1993; Burrows and Pfleger, 2002; Oehl et al., 2003). Similarly, non-mycorrhizal crops in rotation or fallow periods can decrease

and/or delay AMF colonization, sometimes for 60 days, due to the absence of host plants (Gavito and Miller, 1998; Karasawa et al., 2002; Bedini et al., 2007). Tillage is also widely observed to negatively impact colonization by AMF, due to disruption of hyphal networks in the soil (Boddington and Dodd, 2000; Miller, 2000). While organic farming historically relies heavily on conventional tilling practices, many conventional farms in SK have moved towards no till rather than conventional tillage. Farmers employing no till leave more than 30% of crop residues on the soil surface, and use low-impact equipment such as low-disturbance, direct seeders (Peigné et al., 2007). Between 1991 and 2006, surveyed conventional farmers in SK employing no-till methods for seed bed preparation increased 10 to 60% (Statistics Canada, 2007). Therefore tillage is likely less of a negative influence on AMF colonization in conventional than organic systems.

Plant colonization by AMF is typically lower in soils with high P content (Allen et al., 1981; Douds and Schenk, 1990; Vivekanandan and Fixen, 1991), and it can be delayed or decreased with additions of P-inputs (Asimi et al., 1980; de Miranda et al., 1989; Hinsinger, 2001; Ryan and Graham, 2002). Colonization is generally greater in organic than conventional systems, as inputs of P are largely absent (Mäder et al., 2000; Oehl et al., 2003; Entz et al., 2004). A study in Swift Current, SK showed no change in abundance of AMF DNA in soil after P fertilizer addition, however colonization of the alfalfa crop decreased as more inorganic fertilizer P was added (Beauregard et al., 2010). Similarly, Entz et al. (2004) found greater AMF colonization of flax in an organic than conventional system.

The presence or absence of weeds in organic systems plays an important role in crop yields and AMF colonization, and has a range of effects on AMF efficiency. For example, one study reported that benefits from AMF for plant P uptake did not compensate for losses from competition with weeds for nutrients (Galvez et al., 2001). A further study observed that maize

yields increased in the absence of non-mycorrhizal weeds, and that yields decreased with the removal of mycorrhizal weeds (Feldmann and Boyle, 1999). A study examining colonization of highly mycorrhizal plant species in the presence of non-mycorrhizal weeds found that that the non-mycorrhizal weeds either had a negative or no affect on AMF colonization of the host species (Chen et al., 2005). This confirmed results of Fontanela et al. (1999), who noted that AMF colonization of pea was reduced or unaffected by non-mycorrhizal weeds. They observed the greatest reduction in AMF colonization when non-mycorrhizal weed species established before pea (Fontanela et al., 1999).

In the absence of a host plant, AMF exist as spores in soil. There are many techniques to extract the spores, and among the most used are wet-sieving methods (An et al., 1990). These wet-sieving methods will remove the majority of spores from soil, including those that may not be viable, that is, able to colonize plants. In this case, analysis of AMF DNA will provide an indication of the viable and living AMF biomass in a soil (Olsson, 1999). This complete dependence on a host plant is a drawback when studying AMF in a laboratory setting, as no way has yet been discovered to isolate spores and germinate them without a host (An et al., 1990).

2.6 Soil Phosphorus Amendments

Limitations on fertilizer inputs means organic farmers must look for natural and approved solutions for soil fertility management. Due to the relatively unavailable nature of soil P in SK, it often becomes necessary for organic farmers to look beyond cropping rotations when addressing soil fertility. Three allowable amendments for soil P are composted manure, rock phosphate (RP), and bone meal (BM).

2.6.1 Composted manure

On average, 75-90% of major nutrients digested by livestock will pass directly through the animal into the manure (Adler and Sikora, 2003), which makes it an important resource for

all farming systems. Applications of manure are known to increase yield, tissue nutrient concentrations, and plant biomass (Smith et al., 1997; Delschen, 1999; Stamatiadis et al., 1999; Brandt et al., 2007). Availability of nutrients from manure depends upon what is fed to the livestock, storage of the manure, time of application, and animal type (Schoenau et al., 2010). Depending on the type of manure, nutrients such as N, P, potassium (K), or sulfur (S) may not be present in plant-available forms (Schoenau et al., 2010), and will only become available over time as nutrients mineralize. For instance, the N in solid cattle manure is often present only in organic forms, and must mineralize to become plant-available (Qian and Schoenau, 2002). Immobilization and mineralization of N and P in manure is also related to their balance with C (Tisdale and Nelson, 1975).

Organic farmers are allowed the use of composted and non-composted manure. Non-composted manure refers to solid and liquid forms of raw manure (Canadian General Standards Board, 2011). For crops that do not have an edible part in contact with soil (e.g. tomatoes, peas), raw manures must be incorporated into soil at least 90 days before harvest. When edible parts are in contact with soil or soil particles (e.g. carrots, lettuce), incorporation into soil must occur at least 120 days before harvest (Canadian General Standards Board, 2011). Farmers must use manure sourced on-farm or from an organic farming source whenever possible. However, manure can be used from a conventional source if livestock can turn 360° in their pens and are not kept entirely in the dark (Canadian General Standards Board, 2011).

Organic farmers generally use composted manure rather than raw manure, as nutrients in compost are more stable than in raw manure and thought to be less harmful to the environment (Seiter and Horwath, 2004; Walz, 1999). As mentioned previously, however, manure use is limited on SK organic farms due to the large average farm size (Knight et al., 2010a). Livestock

operations are not practical on such large areas. Typically, organic farms with livestock in SK do not have herds large enough to fertilize all of their farmland. Approximately half of organic farms from a survey in SK had cattle on their farms, but only 12% had applied manure to their fields in the past 5 years (Buhler, 2005).

The benefits to soil from composted manure addition have been seen in the long and short term (Smith et al., 1997; Delschen, 1999; Stamatiadis et al., 1999; Carpenter-Boggs et al., 2000; Leifeld et al., 2002). Additions of compost to soil may increase soil microbial biomass (Mäder et al., 2002; Fliessbach et al., 2007) and production of SOM (Fliessbach et al., 2007). Composted manure is manure that has been aerobically decomposed by microorganisms under controlled conditions (Adler and Sikora, 2003). Microbial activity raises the temperature of the compost pile, and the demand for oxygen spikes as elevated decomposition rates occur (Adler and Sikora, 2003). During this process, plant-available N in the compost decreases almost 50% due to conversion of N to organic forms (Adler and Sikora, 2003). This results in plant-available N comprising 0% to 30% of total N in manure compost material (Sikora and Szmidt, 2001). The conversion of N to organic forms narrows the inorganic N:P ratio of the material, making it more likely that P is mineralized after the composting process (Adler and Sikora, 2003).

Unlike N, P is preserved during composting (Eghball and Power, 1999; Eghball et al., 2002). In composted manure sources, 75% to 90% of total P is inorganic P (Eghball et al., 2002), and even composts with very low P concentrations (below 0.01%) have high P availability (Sikora and Enkiri, 2005). Care must be taken when applying composted manure to soil, however. The N:P ratio of compost is often narrower than most plants, which can result in loading of soil P (Douds et al., 1997; Mikkelsen, 2000).

Generally, the benefits of composted manure depend on its maturity. Immature compost will initially increase soil biological activity, while benefits from mature compost are sustained over the growing period (Adler and Sikora, 2003). Biological activity is greatest in immature compost, and levels out as it ages (Adler and Sikora, 2003). The maturity of compost is related to its change in temperature over a specified length of time. If the temperature of compost rises more than 10 °C over 5 days, it is considered immature, and a temperature rise of less than 10 °C over 5 days indicates maturity (Brinton et al., 1995). A study that applied composted manure to soil at different maturity stages found that biological activity and P release were greatest at 2 weeks, when the compost was immature (Adler and Sikora, 2003). Once the compost matured to 8 weeks, biological activity stabilized, and the release of P decreased. At the 8-week stage of maturity, the forms of soluble P were very similar to those from the original source (Adler and Sikora, 2003). They concluded that although P-release from the mature compost (8 week maturity) was decreased compared to the immature compost (2 week maturity), the P was less susceptible to run-off and more valuable as a stable source of P for plant uptake (Adler and Sikora, 2003).

The application of composted livestock manure to organic fields in SK contributes very little overall to SOM pools or soil fertility. Despite its limited use throughout the province, it is still of interest due to its potential as a fertilizer. A study conducted on organically farmed land near Elbow, Scott, and Vonda, SK assessed the effects of varying manure application rates on seed nutrient concentration and plant P uptake (Shirtliffe and Knight, 2006). Aside from the Elbow site, plant P uptake and seed concentrations increased in the second year of application. Another long-term organic farming study conducted in Scott, SK by Brandt et al. (2007) confirmed that composted manure can supply N, P, K, S, and other micronutrients to deficient

soils. Although the nutrients were not immediately plant-available, composted manure has value over time in organic systems, provided it is not over-applied.

2.6.2 Rock phosphate

Rock phosphate (RP) refers to mineral forms of phosphates, and its use is approved in organic systems (Canadian General Standards Board, 2011). It is mined around the world with the largest production in China (Jasinski, 2013). There are many different kinds of mineral phosphates that exist in nature, and their varying characteristics determine their importance in the agricultural sector. The particle size of RP largely determines its solubility and plant-P availability. Generally, the finer the particle size, the more likely that solubilization will occur (OACC, 2007).

Rock phosphate is acceptable according to organic standards, although its performance is found to be erratic in soils such as those found in SK (Shirtliffe and Knight, 2006). Generally, RP will more readily dissolve in soils with pH<6 (Bekele and Hofner, 1993; Surendra et al., 1993). The dissolution of hydroxyapatite (HAP), its main component, requires the presence of H^+ ions in the soil. Dissolution of HAP also depends on soil P and Ca concentrations (Khasawneh and Doll, 1978). Many of the soils used for farmland in SK have pH>6 due to calcium carbonate concentrations from their parent material, which limits the potential of RP in these soils (Pennock et al., 1999; Shirtliffe and Knight, 2006). A study on several organic farming sites in SK found slight increases on overall yields of wheat and pea (883.35 kg ha^{-1} versus 933.14 kg ha^{-1}) after applications of RP to soil (Shirtliffe and Knight, 2006). Despite increases in crop yield, researchers postulated that RP would not produce economic return for organic producers, though RP may provide soil P over the long-term (Shirtliffe and Knight, 2006). Similarly, a further study found no effect from RP application on plant biomass, yield,

plant P concentration, and plant P uptake, unless it was applied in combination with fungal inoculants for P-uptake (Takeda, 2004).

Despite its limited success on SK organic farms, RP may still be an option to replace soil P over the long-term. It is also possible that finely grinding the material may increase its P availability to plants (Knight et al., 2010a). It may also be more effective when combined with other soil amendments.

2.6.3 Bone meal

Bone meal (BM) consists mainly of N, P, and Ca (Jeng et al., 2006). The dissolution of P from BM is similar to RP, as HAP is also present in its composition. Similarly, it has been found that BM dissolution, and therefore its effectiveness as a fertilizer, increases in acid soils compared to soils with pH>6 (Bekele and Hofner, 1993; Surendra et al., 1993).

The use of BM was banned in the European Union in 1994 due to fears of transmissive spongiform encephalopathies (TSE), or mad-cow disease (Kamphues, 2002). More recently, the ban was lifted, and it has been explored as a source of N and P for crops (Novelo et al., 1998; Jeng et al., 2004 and 2006). Studies have shown increases in alkaline phosphatase activity, N mineralization, and microbial activity after the addition of BM to acidic and alkaline soils (Novelo et al., 1998; Mondini et al., 2008).

2.7 Soil Quality

Soil quality refers to the inherent ability of a soil to perform necessary functions, and how these functions are affected by management practices (Bastida et al., 2008). Soil quality is an important aspect of natural systems as well, but for the purposes of this work the definition is focused on agricultural systems. The complexity of soil ecosystems means their health cannot be measured by one factor alone, and soil physical, chemical, and biological properties must all be

taken into account for a complete measurement of soil quality (Rapport, 1989; Constanza et al., 1992). Processes used to measure soil quality should be sensitive to climate and management changes, closely related to ecosystem behaviors, and easily measured in the field (Doran and Parkin, 1994).

For the purpose of organic farming and best soil management practices, this research focused on biological soil properties. They are more sensitive to land management changes, namely agricultural inputs and crop rotation (Bastida et al., 2008).

2.7.1 Microbial biomass phosphorus

Soil microbial biomass (SMB) is defined as the living portion of SOM, excluding flora and fauna and all soil animals larger than 5×10^{-3} μm^3 (Jenkinson and Ladd, 1981). Although SMB comprises less than 5% of SOM, it performs valuable soil functions. Its turnover rate is much faster than organic matter (Jenkinson and Ladd, 1981; Paul, 1984). It is a labile source and immediate sink for C, N, P, and S and plays a vital role in nutrient transformation (Dalal, 1998). Soil microbial biomass is typically larger and more diverse in organic than conventional systems (Stockdale and Watson, 2009), but may be negatively impacted from tillage. A three-year study by Berner et al. (2008) found that microbial biomass carbon (C_{mic}) increased 28% when tillage intensity was reduced in an organic system compared to conventional tillage in a high-input system. The increased use of no-till on conventional SK farms between 1991 and 2006 may therefore positively affect SMB activity in conventional versus organically-managed soils, although other aspects must be taken into consideration (Statistics Canada, 2007; Berner et al., 2008).

The drawback of SMB as an indicator of soil quality is its variability in size and activity across soil types due to factors such as soil texture, climate, mineralogy, seasonal variability, and land use (Grananstein et al., 1987; Srivastava, 1992; Ritz and Robinson, 1988; Tate et al., 1991).

In the context of lab analyses, only meaningful comparisons can be drawn between different treatments of the same soil type, and target baseline values are largely absent.

Microbial biomass P (P_{mic}) refers to the portion of P within SMB, and its activity is often related to the immobilization and mineralization of P in soil (Oberson et al., 1996). Microbial biomass P activity depends on the quantity and quality of substrate added, P_{mic} size, soil properties, and the community of flora and fauna (Oberson et al., 1996). Buenemann (2003) found P immobilization by P_{mic} was proportional to the amount of soluble C added. Initial additions of glucose increased P_{mic} two- to sixfold and the concentration of extractable P drastically decreased. This immobilization of P by P_{mic} has been noted elsewhere (Ofori-Frimpong and Rowell, 1999; Schomberg and Steiner, 1999). It has also been observed that P_{mic} may immobilize P from soil P_i already present in the system (Buenemann et al., 2004). Once microorganisms release P, it may be in the form of P_o (Chauhan et al., 1979), P_i (Oehl et al., 2001b), or be absorbed by growing plant roots (Oberson and Joner, 2005). The cycle of immobilization-mineralization depends upon the degradability of substrates and soil properties (Buenemann, 2003).

Microbial biomass P has been strongly correlated with SOM (Schärer, 2003; Oberson and Joner, 2005). In general, organic cropping systems contain nearly twice as much P_{mic} as conventional systems (Oberson et al., 1996; Oehl et al., 2001b), which may be due to higher levels of SOM (Tuomisto et al., 2012) and the absence of pesticides (Oberson et al., 1993). As discussed previously, however, SOM levels in organic systems may be lower, equal, or higher to SOM levels in conventional systems, depending on the C:N ratio of plant material incorporated into soil and tillage practices. In SK in particular, the lower SOM levels on organic than conventional farms may indicate lower P_{mic} as well. Levels of SOM and P_{mic} will also depend on

the season of soil sampling and the soil moisture levels. Compared to C_{mic} and microbial biomass N (N_{mic}), relatively little research has been done on P_{mic} in the Canadian Prairies. However, it is assumed that P_{mic} will function similarly to C_{mic} and N_{mic}, as the nutrients are all present within SMB.

Green manures can contribute substantially to SMB, due to the substrate added from the living plant material. Levels of C_{mic} were greater from red clover **(***Trifolium pretense* L.) green manure incorporation than pea, canola, wheat, and barley residues in a study in Beaverlodge, AB (Lupwayi et al., 2006a). A study in Swift Current, SK found C_{mic} following green manure incorporation was 50% higher than C_{mic} in a wheat-fallow system and 50-75% higher than C_{mic} in a continuous wheat system (Biederbeck et al. 2005). Although the additions of SOM by legumes may not be substantial, their contributions to SMB may be important in organic systems.

Soil P amendments can increase SMB. Applications of composted manure typically increase SMB, largely due to the stimulation from the organic components (Mäder et al, 2002; Fliessbach et al., 2007). Novelo et al. (1998) and Mondini et al. (2008) observed increases of SMB after additions of BM to acidic and alkaline soils. The relative success of these amendments on SMB across soil types may indicate their use in organic systems on the Prairies, and an overall contribution to soil nutrient cycling.

2.7.2 Phosphatase enzymes

Phosphatase enzymes are measured in soil to quantify potential P_i release from organic sources rather than the current status of P (Dick, 1997). The phosphatase enzyme itself induces chemical transformations that will eventually release orthophosphate from various P_o compounds, and may provide an estimate of the mineralization potential of soil P.

There are two types of phosphatase enzymes in soil, acid and alkaline. Activity of the acid phosphatase enzymes originate from plants, plant roots, and microbial communities (Tarafdar and Marschner, 1994; George et al., 2002), while the activity of the alkaline phosphatase enzyme comes from bacteria and fungi but not from plants (Hebrien and Neal, 1990). It is often positively correlated with SMB (George et al., 2002; Okur et al., 2009). Activity of each is generally higher in organic than conventional systems (Kremer and Li, 2003). Rather than attributing this to the enzymes present in organic amendments, it is most likely due to the stimulation of microbial activity from amendment addition to soil (Martens et al., 1992). For example, Mondini et al. (2008) found a 25% increase in alkaline phosphatase (ALPase) activity after applications of BM to acidic and alkaline soils in a greenhouse study. Activity increased two days into the incubation and stabilized for the remainder of the experiment.

Alkaline phosphatase enzymes (ALPase) have been found in hyphae of AMF (Gianinazzi-Pearson and Gianinazzi, 1978; Tisserant et al., 1993). The highest level of ALPase enzyme activity is seen during AMF colonization, implying that ALPase enzymes may be linked to the assimilation of P by the host plant (Gianinazzi-Pearson and Gianinazzi, 1978; Guillemin et al., 1995; Jabaji-Hare et al., 1990; Tisserant et al., 1993), and that ALPase is a significant contributor to the P nutrient transfer between AMF hyphae and host plant (Tisserant et al., 1993). Therefore ALPase activity may represent an important component of the AMF-host plant symbiosis.

3. SOIL PHOSPHORUS DYNAMICS IN A LONG-TERM ORGANIC FARMING SYSTEM IN SCOTT, SK

3.1 Preface

Many organic farmers in SK face plant available phosphorus (P) shortages due to the binding of P with various cations in soil. Phosphorus is a macronutrient required by all crops that is necessary for early maturity, crop root growth, and development of reproductive organs. Due to restrictions on inputs and the low use of manure on SK organic farms because of small herd and large farm size, P in organic systems is largely supplied for plants through crop rotation. Crop P-uptake is also enhanced by naturally-occurring arbuscular mycorrhizal fungi (AMF). Soil microbial processes are also important when assessing soil P, as they are sensitive to land management changes and reflect transformations of P in soil. The field experiment described in this chapter quantifies soil P dynamics and AMF colonization under four cropping sequences under long-term organic management. This is the first of two experiments in this thesis regarding soil P on organic farms, and unlike the second does not involve the use of P fertilizer amendments.

3.2 Abstract

Organic farms in SK are often deficient in plant-available P, and the use of livestock manure to supply P for plant uptake is limited. While many organic farmers have cattle, herds are often not large enough to supply manure for the entire farm. This means organic farmers must rely on crop rotation and naturally-occurring AMF to provide P for plant growth. Colonization by AMF in conventional systems is often negatively affected by non-mycorrhizal (non-host) crops in rotation, fallow periods, or additions of soluble P fertilizers.

Field sampling was carried out in the 2012 growing season to examine the effects of crop rotation on soil P dynamics, plant P and N uptake, and AMF colonization of various crops in a long-term organic system. Crops were sampled following a non-AMF host crop, a partial fallow period, and AMF host crops. Numbers of AMF spores in soil did not decrease and AMF colonization was not delayed by the non-host crop or the partial fallow period. Although not directly measured, soil N enrichment from crop rotation and low soil P_i may have been more influential on AMF colonization than non-host crops or the partial fallow period. Crop rotation also influenced microbial P and plant P and N, as additions of C from lentil plough down dictated mineralization and immobilization levels of soil P by the soil microbial biomass and the levels of N and P in soil available for uptake by plants.

3.3 Introduction

Crop rotations are an important component of organic farming systems in SK and elsewhere, as their proper use will maintain long-term soil fertility for crop growth (Stockdale et al., 2001; Akter et al., 2004). Properly combining nutrient demanding crops with soil-building crops ensures that soil fertility is constantly replenished as needed (Stockdale et al., 2001).

Arbuscular mycorrhizal fungi (AMF) are important components of organic farming systems because of their ability to enhance plant P uptake through the extension of host plant

root systems (Bolan, 1991; Smith and Read, 1997). They rely entirely on host crops for their C supply, and exist as spores in soil when no host plant is present (An et al., 1990). Many factors affect AMF colonization of crops. In conventional farming systems, the presence of a fallow period or a non-mycorrhizal crop (i.e. crops that do not support AMF colonization) will often delay the colonization of a mycorrhizal crop following it (Gavito and Miller, 1998; Karasawa et al., 2002; Bedini et al., 2007). This is due to the decrease of AMF spores in the absence of a host plant (Bedini et al., 2007).

Farm management practices affect AMF colonization of crops. Monocultures decrease colonization due to the lack of host crop diversity (An et al., 1993; Burrows and Pfleger, 2002; Oehl et al., 2003), and tillage disrupts AMF hyphal networks, which also decreases crop colonization (Boddington and Dodd, 2000; Miller, 2000). Levels of P in soils are negatively correlated to AMF colonization (Allen et al., 1981; Douds and Schenk, 1990; Vivekanandan and Fixen, 1991), and similarly, additions of soil P amendments will decrease AMF colonization of crops (Asimi et al., 1980; de Miranda et al., 1989; Hinsinger, 2001).

Activity of AMF is also related to the host crop's root system. Coarse roots, like those found in pea, are more dependent on the AMF symbiosis than fine roots like those of wheat, due to their limited ability to reach immobile pockets of soil P (Plenchette et al., 1983; Paul and Clark, 1989). Mycorrhizal dependency is defined by Plenchette et al. (1983) as the difference between the dry mass of the mycorrhizal crop (grown in unfumigated soil) and the dry mass of the non-mycorrhizal crop (grown in fumigated soil) expressed as a percentage of the dry mass of the mycorrhizal plant. The study found that wheat had 0% mycorrhizal dependency and pea had 96.7% mycorrhizal dependency. They concluded that pea rely as heavily on AMF for P as on N-fixing bacteria for N, whereas wheat does not require AMF for P uptake (Plenchette et al., 1983).

Due to the lack of available soil P and the prohibited use of inorganic fertilizer P in organic systems in SK, the AMF symbiosis may be especially important for organic producers. Crop colonization by AMF is generally higher in organic than conventional systems (Mäder et al., 2000; Oehl et al., 2003; Entz et al., 2004), although the reliance on tillage by many organic farmers can negatively impact AMF communities (Boddington and Dodd, 2000).

When assessing AMF colonization, it is important to consider both potential and realized activity. The abundance of AMF spores in soil, often reported as spore density (SpD, number of AMF spores 100 g soil^{-1}), is an indicator of potential AMF activity in soil. Extraction of AMF spores gives researchers a general idea of potential AMF colonization, as not all spores extracted are viable (An et al., 1990). The use of PLFA analysis extracts the AMF DNA biomarker 16:1ω5c from soil, which gives a more accurate depiction of AMF activity as it represents the living and viable portion of AMF DNA (Olsson, 1999). Realized AMF activity is seen in the colonization of host crops. It has also been noted that the metabolism of AMF may be linked to activity of the ALPase enzyme (Gianinazzi-Pearson and Gianinazzi, 1978; Tisserant et al., 1993). It is possible that ALPase enzymes are closely involved with P nutrient transfer between AMF hyphae and the host plant, and that they also represent realized AMF activity (Gianinazzi-Pearson and Gianinazzi, 1978; Jajabi-Hare et al., 1990; Guillemin et al., 1995).

Microbial parameters are important when assessing soil quality (Bastida et al., 2008). In agricultural systems, soil quality refers to the effect that land management practices have on a soil's ability to perform necessary functions such as nutrient mineralization and immobilization (Bastida et al., 2008). Net immobilization of P will generally occur if C:P>300, and C:P<200 results in net mineralization. Ratios of C:P between 200 and 300 do not result in a dominance of either process (Tisdale and Nelson, 1975). Soil organic matter (SOM) has a C:N:P ratio of

100:10:1, and limited N or P induces mineralization of the other (Tisdale and Nelson, 1975). Therefore soil N must also be considered when measuring soil P.

Soil microbial biomass (SMB) is the living component of SOM (Jenkinson and Ladd, 1981). It has a higher turnover rate than SOM, and plays an important role in mineralization and immobilization of nutrients such as P (Jenkinson and Ladd, 1981; Paul, 1984). Microbial biomass P (P_{mic}) is the amount of P within SMB (Oberson et al., 1996). The activity of P_{mic} in soil is closely related to P immobilization and mineralization, and therefore an important component of plant P nutrition (Oberson et al., 1996). The ALPase enzyme is also related to soil P turnover (Dick, 1997). Its activity is to mineralize P in soil, rendering it available for plant uptake (Dick, 1997). Along with AMF activity, microbial P activity is important when assessing soil and plant P nutrition.

An experiment was conducted to analyze four cropping sequences under long-term organic management. Wheat (W, mycorrhizal) was sampled following a non-mycorrhizal crop (mustard, M) and a partial fallow period (lentil green manure, LGrM). Pea (PP, mycorrhizal) and barley (B, mycorrhizal) were sampled following wheat. Experimental objectives were (i) quantifying soil P dynamics and plant N and P uptake from the four cropping sequences, (ii) determining if AMF SpD, abundance of viable AMF spores, and AMF colonization of mycorrhizal crops decreased after mustard (non-mycorrhizal) and a partial fallow period (LGrM), and (iii) determining if mustard and LGrM delayed colonization of following mycorrhizal crops.

3.4 Materials and Methods

3.4.1 Site description and experimental design

This study was conducted at the Agriculture and Agri-Food Canada (AAFC) Research Farm at Scott, SK (52°24’ N, 108°48’ W) within the Alternative Cropping Study (ACS)

experiment. The ACS experiment is an ongoing study assessing the effects of different cropping sequences, diversity and input levels. The experiment encompasses an organic, a high-input, and a reduced-input system, and in 2012 was in its 18th year. For this experiment only select plots from the organic system were sampled. Plots were 12.8 x 40 m and organized in a randomized complete block design. There are three diversity levels, six rotation phases, and four replicates of each rotation for a total of 144 plots. The six rotation phases are present every year.

Diversity levels are identified by the types of crops in rotation. Low diversity (LOW) is comprised entirely of annuals, DAG (diverse annual grain) is named for the dominance of annual grains, and DAP (diverse annual perennial) is named for the combination of annuals and perennials. Soil and crops were sampled from Phase 2 of LOW, Phase 3 of DAG, Phase 2 of DAP, and Phase 3 of DAP (Table 3.1). For a complete description of soil sampling, refer to Section 3.4.2, and for crop sampling, refer to section 3.4.5.

Table 3.1. Sampled plots from AAFC-ACS† at Scott, SK, 2012. All crop phases are present every year. Samples were taken from each diversity level (LOW=low diversity, DAG=diverse annual grain, DAP=diverse annual perennial) of the organic system. The names of diversity levels refer to the types of crops in their rotations. Sampled crops are bold and in italics.

Input Level	Phase 1	Phase 2	Phase 3	Phase 4	Phase 5	Phase 6	Diversity Level
Organic	LGrM†	***Wheat***	Wheat	LGrM	Mustard	Wheat	LOW
	LGrM	Wheat	***Pea***	Barley	LGrM	Mustard	DAG
	Mustard	***Wheat***	***Barley***	Alfalfa	Alfalfa	Alfalfa	DAP

†AAFC-ACS= Agriculture and Agri-Food Canada, Alternative Cropping Study
‡LGrM=lentil green manure. LGrM was ploughed under at mid-season.

3.4.2 Soil sampling and analyses

Soil from AAFC-ACS is classified as an Orthic Dark Brown Chernozem of the Biggar Association (Ayres et al., 1985) and has a loamy texture (sand=31%, silt=42%, clay=27%, Kirkland and Johnson, 2000). Soil was sampled from Phase 2 of LOW, Phase 3 of DAG, Phase 2 of DAP, and Phase 3 of DAP. Four replicates of four plots were sampled for a total of 16 plots.

Soil samples for soil characterization (Section 3.4.2.1), AMF spore extraction (Section 3.4.2.2), and a bioassay (Section 3.4.4) were collected from the 0 to 15 cm Ap horizon of each plot with a Dutch auger (5 cm diam x 15 cm depth) at pre-seeding (May 9, 2012). Four to five soil samples were composited for each measurement from each plot, placed field-moist in plastic bags, and stored in an ice-filled cooler on site. Upon return to the laboratory, samples for soil characterization and AMF spore extraction were stored in a 4 °C refrigerator until analysis, and bioassay soils were sieved to 2 mm and air-dried until used in the greenhouse.

Five 0 to 10 cm depth increment soil samples from each plot were taken for phospholipid fatty acid (PLFA) analysis (Section 3.4.2.3) with a Dutch auger (5 cm diam) at pre-seeding (May 9, 2012) and flowering (July 11, 2012) and composited by plot and date. Samples were taken at this depth to sample the most microbiologically active portion of the soil profile (Bligh and Dyer, 1959). Field moist soil samples were placed in 15 mL BD falcon tubes (BD Biosciences, San Jose, CA) and stored in an ice filled cooler on site. Once in the laboratory, samples were transferred to a -80 °C freezer and stored until analysis.

Soil for ALPase (Section 3.4.2.4) was collected from the Ap horizon at pre-seeding and flowering with a Dutch auger (5 cm diam x 15 cm depth) and composited by plot and date. Five samples were taken at each date. On site, soil samples were placed field moist in plastic bags and stored in an ice filled cooler. After returning to the laboratory, samples were stored at 4 °C until analysis.

Five soil samples for P_{mic} (3.4.2.5) were taken from the surface (0 to 5 cm) of each plot with a Dutch auger (5 cm dia) at pre-seeding and flowering, composited by date, and stored field moist in a cooler in plastic bags until return to the laboratory. At the laboratory, P_{mic} samples were stored at 4 °C until use.

3.4.2.1 Soil characterization

Soil characterization measurements of pre-seeding soil were performed by technical staff in the Department of Soil Science, University of SK. Soil moisture content (SMC) of each sample was determined by measuring 10 g field-moist soil in a pre-weighed aluminum dish and heating for 24 h at 105 °C to reach a stable oven-dry weight. Once cooled, the dry soil was re-weighed, and SMC (kg water kg^{-1} soil) was determined via the following equation:

$$SMC = \frac{\text{wet soil weight (g)} - \text{dry soil weight (g)}}{\text{dry soil weight (g)}} \times 100 \qquad \text{(Eq. 3.1)}$$

Soil inorganic N (NO_3-N, µg g^{-1}) was extracted via 2.0 *M* KCl extraction (Keeney and Nelson, 1982). For each sample, 5.0 g field-moist soil was weighed into a 125 mL Erlenmeyer flask, and 50 mL of 2.0 *M* KCl solution was added to the sample. Flasks were stoppered and shaken on a rotary shaker (New Brunswick Scientific G10 Gyrotory © Shaker, Edison, NJ) for 30 min at 160 rpm and filtered through filter paper #454 (VWR International LLC, Radnor, PA) into 7 dram vials. Nitrate (NO_3^-) and ammonium (NH_4^+) concentrations of the filtrate were analyzed on the Technicon Autoanalyzer II (Technicon Industrial Systems, Tarrytown, NY, 1973).

Soil P_i (available phosphate P, µg g^{-1}) was extracted via Kelowna extraction (Qian et al., 1994). For analysis, 3.00 to 3.09 g of dried soil from each sample were weighed into 125 mL Erlenmeyer flasks, and 30 mL Kelowna solution (28 mL acetic acid, 38.5 g ammonium acetate,

1.11 g ammonium fluoride dissolved in 2 L distilled water) added. Flasks were stoppered and shaken horizontally for 5 min at 160 rpm on a rotary shaker (New Brunswick Scientific, G10 Gyrotory © Shaker, Edison, NJ). The solution was filtered through filter paper #454 (VWR International LLC, Radnor, PA) into 7 dram vials, and measured for PO_4^{-3} content on the Technicon Autoanalyzer II.

Soil pH was determined on a 2:1 water:soil extract per Hendershot et al. (2008). Twenty mL of deionized water was added to 10 g of each soil sample. Samples were stirred intermittently for 30 min then allowed to settle for 1 h. The pH of the water-soil solution was read by a pH meter (Fisher Scientific Accumet pH meter, Singapore).

3.4.2.2 Arbuscular mycorrhizal fungi spore extraction

Spores were extracted from each soil sample via the wet-sieving method of An et al. (1990). To prevent killing spores, tap water was used throughout. Twenty g of air-dried soil was suspended in 150 mL water and mixed with a soil stirrer (Hamilton Beach Classic Black Drinkmaster Drink Mixer 729) for 5 to 10 min, and the resulting mixture was sieved to retain the soil fraction between 53 µm and 425 µm. This fraction was resuspended in water to repeat the procedure. Samples were washed into 50 mL centrifuge tubes, suspended in water, and centrifuged (Sorvall SE 6+ Centrifuge, Thermo Scientific, Waltham, MA) for 5 min at 1270 x g. The supernatant was decanted and discarded. Centrifugation with tap water removes organic debris. The pellet was resuspended in 50% sucrose and centrifuged for 5 min at 1270 x g. Centrifugation with sucrose extracts spores from the soil. The supernatant containing the sucrose and AMF spores was transferred to 7 mL vials and filtered through 55 mm filter paper. Spores were washed with water to remove any remaining sucrose. Spores were washed onto a Petri dish (100 x 15mm) with tap water and counted under a stereomicroscope (Stemi SV 11, Carl Zeiss

Canada, Don Mills, ON). Finally, spore counts were multiplied by 5 to obtain AMF spore density (SpD) per 100 g soil.

3.4.2.3 Phospholipid fatty acid analysis

Phospholipid fatty acid (PLFA) analysis was performed via the method of White et al. (1979), modified from Bligh and Dyer (1959). The biomarker 16:1ω5c was used to identify AMF biomass (Olsson, 1999). After storage in a -80 °C freezer (New Brunswick Scientific Ultra Low Temperature Freezer, U535 Innova, Edison, NJ), soils were freeze-dried for 48 h (VirTis Wizard 2.0 Lyophilizer Controller, SP Industries Inc., Warminster, PA). Four g soil from each freeze-dried sample were hand-ground to 2 mm with a mortar and pestle, weighed, and placed into 50 mL Kimax tubes (VWR Kimax tubes, VWR International LLC, Radnor, PA).

To extract fatty acids, an extractant solution was made consisting of 220 mL methanol (MeOH), 110 mL chloroform ($CHCl_3$), and 88 mL phosphate buffer (2.18g potassium phosphate (K_2HPO_4) in 250 mL ultrapure water and 0.75 mL $CHCl_3$). Fifteen mL of extractant solution was added to each tube containing the 4 g freeze-dried soil samples. Samples were covered with aluminum foil, shaken for 2 h at 25 °C on a rotary shaker (G24 Environmental Incubator Shaker, New Brunswick Scientific Co., Inc., Edison, NJ), and centrifuged (Beckman Model TJ-6 Centrifuge, Beckman Instruments, Inc., Fullterton, CA) for 10 min at 137.4 x g . Following centrifugation, the supernatant from each sample was decanted into a second set of clean 50 mL tubes containing 5 mL phosphate buffer (2.18g K_2HPO_4 in 250 mL ultrapure water and 0.75 mL $CHCl_3$). Once samples were transferred, 4 mL $CHCl_3$ was added to each tube, briefly vortexed (VWR Analog Vortex Mixer, VWR International LLC, Radnor, PA), then covered with aluminum foil for 10 to 15 min to allow the polar and non-polar layers to separate.

Once separated, the non-polar bottom layer was transferred with a 9-inch Pasteur pipette (Fisherbrand™ Pasteur Pipets, Fisher Scientific, Ottawa, ON) to clean 8 mL Kimax tubes and

the top layer discarded. Methanol was added to any cloudy samples (non-polar layer) with an eyedropper and vortexed until clear. One mL of MeOH was added to each tube, and samples were dried down completely on an N_2 evaporator (Techne Dri-Block DB 3, Bibby Scientific Limited, Staffordshire UK) at 30°C. The addition of MeOH and evaporation was repeated once. Samples were wrapped in aluminum foil and stored overnight at -20 °C.

Following fatty acid extraction, the fatty acids were separated using solid phase extraction (SPE) columns (Varian Inc., Mississauga, ON). Each column was conditioned with 3 mL $CHCl_3$. Samples from the previous day were resuspended with the addition of 1 mL $CHCl_3$, and after complete drainage of $CHCl_3$ from the columns, the contents of each sample was transferred from the 8 mL Kimax tubes to the SPE columns with a 5-inch glass Pasteur pipette.

Five mL $CHCl_3$ was added to the SPE columns and drained into 8 mL waste tubes. This fraction was discarded. Following this step, two aliquots of 5 mL acetone were added to each SPE column, drained into the 8 mL waste tubes, and discarded. The 8 mL waste tubes were removed and a fresh set of 8 mL Kimax tubes was placed under the SPE columns. Five mL MeOH was drained through each SPE column, captured in the fresh set of tubes, and dried down with the N_2 evaporator. Samples were covered with aluminum foil and stored overnight at -20 °C.

Lipids within the samples were redissolved the following day in 1 mL 1:1 MeOH:toluene and 1 mL methanolic KOH solutions (0.36 g KOH dissolved in 30 mL MeOH). Samples were vortexed for 10 s then incubated for 15 min in a 35°C water bath. After samples had cooled to room temperature, 2 mL of 4:1 hexane:$CHCl_3$ solution was added to each.

Each sample was neutralized with the addition of 0.1 to 0.25 mL acetic acid mixture (4.60 mL acetic acid and 80 mL ultrapure water) and vortexed for approximately 5 s. Sample pH

was then adjusted to 6 to 8 with the methanolic KOH solution and the acetic acid mixture. Then 2 mL ultrapure water was added to each and samples were centrifuged for 5 min at 137.4 x g.

After centrifuging, the supernatant was decanted and placed in a clean set of 4 mL vials. Ten µL of the 0.1 µL L^{-1} nondecanoic acid methyl ester standard was added to each. The remaining sample in the 8 mL Kimax tubes was centrifuged, and the supernatant transferred to the corresponding 4 mL vials. Following the second centrifugation, samples were covered with aluminum foil and dried down on the N_2 evaporator. They were stored overnight at -20 °C.

To prepare for analysis, samples were resuspended the following day in 150 µL hexane and transferred to 2 mL amber glass vials with plastic inserts and lids. The lids were securely crimped on the vials once each sample was transferred. Finally, samples were stored in a -20 °C freezer until analysis.

Samples were analyzed on a gas chromatograph with an Agilent Ultra 2 cross-linked 5% PH ME Siloxane column (23.85 m x 0.2 mm ID x 0.33 µm film thickness) and a flame ionization detector (FID) (Hewlett Packard 5890 Series II Gas Chromatograph, Mississauga, ON). The oven temperature was ramped from 170 °C to 260°C at 5 °C min^{-1} then to 310 °C at 4 °C min^{-1} increments with a run time of 20.75 min. The injector temperature was 250 °C and the detector temperature was 300 °C. Analysis was based on the identification of peaks by fatty acid standards and MIDI identification software (Microbial ID Inc., Newark, DE) and the addition of the nondecanoic acid methyl ester standard.

3.4.2.4 Alkaline phosphatase enzyme activity

Alkaline phosphatase enzyme activity was measured via the method outlined by Tabatabai and Bremner (1969) with a modified universal buffer (MUB) described by Skujins et al. (1962). A pH of 8 was chosen for the MUB because it is the pH optima for ALPase activity (Ross et al., 1951).

For the MUB, a stock solution consisting of 3.025 g tris (hydroxymethyl) aminomethane, 2.90 g maleic acid, 3.50 g citric acid, 1.57 g boric acid, and 122 mL 1*M* sodium hydroxide (NaOH) were mixed and adjusted to 250 mL with deionized water. To 20 mL of the stock solution, 10 mL of 0.1 *M* NaOH was added, and adjusted to pH 8 with 3 *M* NaOH. The mixture was brought to a final volume of 100 mL with deionized water.

Soil was air-dried for 2 wks then hand-ground with a mortar and pestle to 2 mm. One g soil for each sample was measured into 50 mL Erlenmeyer flasks. Four mL of MUB, 0.25 mL toluene, and 1 mL 0.115 *M p*-nitrophenyl (PNP) solution were added to each sample and swirled to mix the contents. The flask was stoppered and contents incubated for 1 h at 37 °C (VWR Shaking Incubator, VWR International LLC, Radnor, PA). Following incubation, 1 mL of 0.5 *M* calcium chloride (CaCl) and 4 mL 0.5 *M* NaOH were added to each flask, swirled to mix, and filtered through a Whatman^{TM} No. 1 folded filter paper (Fisher Scientific, Ottawa, ON). Controls for each soil involved the addition of 1 mL PNP solution after the additions of CaCl and NaOH.

The concentration of P_i in samples was measured as the degradation of PNP to *p*-nitrophenol over the incubation period. The concentration of *p*-nitrophenol in the filtrate was calculated by referencing a calibration graph. To generate the calibration graph, the optical density (OD) of standards with concentrations of 0 to 50 µg *p*-nitrophenol mL^{-1} deionized water were read on a spectrophotometer **(**DU-6 UV-Visible Spectrophotometer, Beckman Instruments, Inc., Fullterton, CA) at 420 nm and plotted on the y-axis, and concentrations of *p*-nitrophenol were plotted on the x-axis. A linear equation was developed from the graph. The OD values of each sample were read on the spectrophotometer, graphed, and concentrations of *p*-nitrophenol were calculated from the equation of the calibration graph. Units are reported in µg PNP released g $soil^{-1}$ h^{-1}.

3.4.2.5 Microbial biomass phosphorus

Microbial biomass P was measured via the chloroform fumigation-extraction method outlined by Voroney et al. (2007). Soils were sieved to <2 mm and incubated at 40 to 50% field capacity (FC) for approximately 1 wk before the procedure to induce and sustain microbial activity.

To determine FC, cheesecloth was taped to one end of a plastic cylinder (5 x 15 cm). Ten g field moist soil was weighed into the cylinder, and the cylinder was placed upright in a bucket of sand. The soil sample was saturated with water and Parafilm ® was fitted over the open end of the cylinder. The Parafilm ® prevented water loss through evaporation and the cheesecloth allowed the drainage of water without the loss of soil. After 48 h, the soil sample was re-weighed. Then the drained soil sample was heated at 105 °C for 48 h and weighed again to determine water loss. The mass of water lost was determined as the amount of water needed for 100% FC (Eq. 3.2). This equation was multiplied by 0.45 to obtain 45% FC. An average of three samples was calculated for each plot.

$$100\%\ FC = \text{drained soil sample (g)} - \text{dry soil sample (g)} \qquad \text{(Eq. 3.2)}$$

Following incubation, SMC was calculated to determine the oven-dry weight of each sample. Moist soil samples of 15 g were weighed in triplicate and oven-dried (Fisher Isotemp© Oven 200 Series, Model 230F, Thermo Fisher Scientific Ltd, Waltham, MA) for 24 h at 105 °C. Soil moisture content was calculated via equation 3.1. An average of three samples was calculated.

For P_{mic} analysis, nine replicates of approximately 10 g of oven-dried equivalent soil were weighed into 50 mL beakers. Three replicates were fumigated with $CHCl_3$ vapour for 24 h

and six replicates were kept in a sealed dessicator with soda lime for 24 h. For the fumigation procedure, a beaker with approximately 50 mL $CHCl_3$ was placed in a dessicator and surrounded by the soil samples for fumigation. The walls of the dessicator were lined with wet paper towels to minimize changes in the SMC of each sample. The dessicator was vacuum sealed with the aid of vacuum grease and a vacuum pump (Hyvac 14, Central Scientific Company, Cenco Instruments Corporation, Chicago, IL) and fumigated for approximately 5 minutes. Samples were left sealed in the dark for 24 h following fumigation.

The three fumigated replicates and three of the unfumigated replicates (controls) of each sample were extracted with 200 mL of pH 8.5 0.5 *M* NaOH solution and 1 mL deionized water, and the remaining three unfumigated replicates were extracted with 200 mL of pH 8.5 0.5 *M* NaOH and 1 mL P_i spiking solution (250 µg L^{-1} KH_2PO_4). All replicates were shaken on a rotary shaker (New Brunswick Scientific G10 Gyrotory © Shaker, Edison, NJ) at 150 rpm for 30 min and filtered through Whatman™ 42 filter paper (Fisher Scientific, Ottawa, ON).

Microbial biomass P was estimated from the determination of P_i from the filtered samples, measured via the method of Murphy and Riley (1962). Ten mL of each sample was acidified to pH 1.5 with the addition of 6 mL 0.9 *M* H_2SO_4 and stored in the refrigerator for 30 min. Samples were centrifuged for 10 min at 25,000 x g at 0 °C (Sorvall SE 6+ Centrifuge, Thermo Scientific, Waltham, MA) and the supernatant transferred to 50 mL flasks. To adjust sample pH, a few drops of 10% *p*-nitrophenol solution (w/v) were used as an indicator, followed by additions of 4 *M* NaOH and 0.25 *M* H_2SO_4 until the solution was clear. Samples were prepared for analysis with the addition of 8 mL of a colour developing solution (250 mL 2.5 *M* H_2SO_4, 75 mL 0.03 *M* ammonium molybdate solution, 50 mL 0.30 *M* ascorbate solution, and 25 mL 0.019 *M* antimony potassium tartrate solution).

For the standard curve, the P_i spiking solution used previously was diluted to a concentration of 10 µg P_i L^{-1} and 8 mL colour developing solution was added. The 10 µg P_i L^{-1} solution was diluted to 2, 4, 6, and 8 µg P_i L^{-1} in deionized water for use as standards. Deionized water was also used for the blank (the 0 µg P_i L^{-1} standard). Standards were read at 712 nm on a spectrophotometer (DU-6 UV-Visible Spectrophotometer, Beckman Instruments Inc., Fullterton, CA). The optical density (OD) of each standard was plotted on the y-axis, and the P_i concentration of each was plotted on the x-axis. A linear equation was developed. The P_i concentration of each sample was determined by reading the OD value on the spectrophotometer and plotting it in the linear equation.

The P_{mic} of each sample was calculated through a series of equations. The oven-dry equivalent mass of the soil sample (MS) was determined:

$$MS\ (g) = \frac{\text{soil wet weight (g)} \times 100}{(100 + SMC)} \qquad \text{(Eq. 3.3)}$$

From this, the total volume of solution in extracted soil (VS) was calculated using Equation 3.4**:**

$$VS\ (mL) = \frac{\text{(soil wet weight (g)-soil dry weight (g))}}{1\ g\ mL^{-1}} + \text{extractant volume (mL)} + 1\ mL\ (H_2O \text{ or } P_i) \qquad \text{(Eq. 3.4)}$$

Total weight of extractable P_i in samples spiked with P_i was calculated as:

$$P_i \text{ spiked soil } (\mu g\ g^{-1} \text{ soil}) = P_i \text{ spiked soil } (\mu g\ mL^{-1}) \times \frac{(VS\ (mL))}{MS(g)} \qquad \text{(Eq. 3.5)}$$

Finally, P_{mic} was calculated in Equation 3.6. Phosphorus in the fumigated soil is denoted by P_F, P_{UF} denotes P in the unfumigated soil, k_{EP} = 0.4 (Voroney et al., 2007) and is the efficiency of P_{mic} extraction, and R= 100 [(P_i spiked soil – soil P_{UF}) / P_i spike], which is the percent recovery of the P_i spike (Voroney et al., 2007). The P_i spike = 250 µg P_i L^{-1}.

$$P_{mic}\ (\mu g\ g^{-1}\ soil) = \frac{(P_F - P_{UF})}{K_{EP}} \times \frac{100}{R} \qquad \text{(Eq. 3.6)}$$

3.4.3 Root sampling and analysis

Roots were sampled on June 21, July 5, and August 15. Five crop samples were hand-harvested from each plot on each date. Refer to section 3.4.5 for a complete description of above-ground crop sampling. Soil was excavated to 15 cm in crop rows and roots were gently shaken to remove soil. Upon return to the laboratory, roots were separated from shoots at soil surface with scissors and washed with tap water. Roots were cut into 1 cm segments and stored in a 50% ethanol solution at 4 °C until stained for AMF colonization.

Roots were measured for percentage AMF colonization via the ink and vinegar staining technique outlined by Vierheilig et al. (1998) and any stained AMF structures (i.e. hyphae, vesicles) were counted via the gridline intersection method (Giovannetti and Mosse, 1980). After storage in the ethanol solution, all roots were placed in cassettes (Fisherbrand™ Histosette II™ Tissue and Biopsy Cassettes, Fisher Scientific, Ottawa, ON) and boiled in 2 *M* KOH solution until they appeared clear. Boiling times ranged from 10 to 30 min, and varied with root age. Youngest roots were boiled the least. Roots were rinsed with tap water and boiled for 3 min in a staining solution containing 5 mL blank ink (Sheaffer Skrip Black fountain pen ink, The Goulet Pen Company, Ashland, VA) and 100 mL household vinegar. After staining, roots were gently

rinsed over a 500 mL beaker to capture the acidified tap water until water ran clear. Then roots were submerged in the acidified tap water for 20 to 30 min to set the colour.

To analyze and count AMF structures, a grid was made on a Petri dish (100 x 15mm) with 100 intersections spaced 0.5 cm apart. Stained roots were randomly dispersed on the Petri dish with a small amount of distilled water to hold them in place. Roots did not overlap. Each stained AMF structure present at a grid intersection was recorded as 1% mycorrhizal infection. Structures of AMF were identified with use of a stereomicroscope (Stemi SV 11, Carl Zeiss Canada, Don Miles, ON).

3.4.4 Bioassay for arbuscular mycorrhizal fungi colonization potential

A bioassay was conducted to measure the AMF colonization potential from the field soil. Its purpose was to examine AMF colonization as a function of the field soil rather than as a function of differences between individual crops. It was based loosely on the bioassay of Mäder et al. (2000), but plants were harvested after 8 wks and the soil was not altered once taken from the field. Soil for the bioassay was collected from each plot used in the field study (4 reps of 4 plots, 16 plots total).

The bioassay was conducted in the U of S greenhouse. Day and night temperatures were 24 °C/21 °C and the photoperiod was 16 h. Field capacity was determined as described in section 3.4.2.5. All wheat plants were maintained at 65% FC, which was determined by multiplying Eq. 3.2 by 0.65. An average from three replicates was calculated.

Coffee filters were placed in the bottom of 16 cone-tainers (2.5 cm dia x 12 cm depth) (Stuewe and Sons Inc., Tangent, OR) to allow water drainage but prevent soil loss. Cone-tainers were labeled with plot number from AAFC-ACS and placed upright in a tray (61 x 30 x 18, 98 cell capacity) (Ray Leach trays, Stuewe and Sons Inc., Tangent, OR). Forty g soil from each plot of the field study was weighed into each cone-tainer, and three wheat seeds (*Triticum aestivum*

L. cv. Lillian) were hand planted 2 cm deep in each. Cone-tainers were arranged in a complete randomized design that was rearranged weekly. Wheat was watered twice weekly and weed seedlings were removed daily.

Roots and above-ground biomass of all wheat plants were hand-harvested after 8 wks. The roots were separated from the shoots with scissors, stored at 4 °C in a 50% ethanol solution, and measured for %AMF colonization via the ink and vinegar staining technique of Vierheilig et al. (1998) which was described in Section 3.4.3.

3.4.5 Above-ground plant sampling and analysis

Wheat (*Triticum aestivum* L., cv. Lillian), barley **(***Hordeum vulgare* L., cv. CDC Copeland) and pea **(***Pisum sativum* L., cv. CDC Meadow) were seeded at rates of 134 kg ha^{-1}, 112 kg ha^{-1}, and 235 kg ha^{-1} at 18 cm row spacing with an International 6200 double disc press drill (Frontline Ag LLC., Dillon, MT). The site was seeded on May 11 to 20, 2012 by AAFC staff.

Maturity of wheat and barley was measured by the Zadoks scale (Zadoks et al., 1974). Five plant samples (roots and aboveground biomass) were hand-harvested randomly from each plot on June 21 (tillering stage or Zadoks 21-29 for wheat and barley, second node vegetative stage for pea), July 5 (heading stage or Zadoks 50-59 for wheat and barley, flowering for pea), and August 15 (dough development stage or Zadoks 83-87 for wheat and barley, maturity for pea), 2012. Root sampling is described in Section 3.4.3. Barley was harvested on September 4 with a Kincaid 8-XP combine (Kincaid Equipment Manufacturing, Haven, KS), wheat was harvested on September 10 with a Kincaid 8-XP combine, and pea was swathed on August 18 with a 15’ Versatile swather (Buhler Versatile, Inc., Winnipeg, MB) and harvested on August 27 with a Kincaid 8-XP combine. All crops were swathed and harvested by AAFC staff. Weed biomass was collected from each plot by AAFC staff with the final harvest of each crop.

Mycorrhizal wheat was sampled following mustard (non-mycorrhizal, M-W) and lentil green manure (partial fallow, LGrM-W), and mycorrhizal pea and barley were sampled following wheat (W-P, W-B). Wheat from LGrM-W was sampled from LOW (Phase 2), pea from W-P and wheat from M-W were sampled from DAG (Phase 3 and Phase 2), and barley from W-B was sampled from DAG (Phase 2).

Upon return to the laboratory, shoots were separated from roots by cutting at root level with scissors. Refer to section 3.4.3 for a complete description of root sampling. Aboveground crop biomass was placed in a paper bag and dried at 105 °C for 24h, ground to 2 mm with a Wiley Mill (Thomas Scientific, Swedesboro, NJ), and measured for N and P concentrations (µg g^{-1}) via the method of Thomas et al. (1967). Plant samples of 0.25 to 0.30 g were weighed into 75 mL digestion tubes. For each block of tubes (40 total), two were used as blanks (no plant material added) and two containing 0.03 g glycine were used as a check for N recovery.

Five mL concentrated sulfuric acid (18 *M* H_2SO_4) was added to each tube and briefly vortexed (VWR Analog Vortex Mixer, VWR International LLC, Radnor, PA). Samples were heated for 30 min on a heating block at 360 °C (SmartDigest^{TM} 20/40, Westco Scientific Instruments, Inc., Brookfield, CT). After cooling for 20 min, 2 mL hydrogen peroxide (H_2O_2) was added to each sample and heated for 30 min. Additions of H_2O_2 and heating cycles were repeated until samples were clear. The number of heating cycles varied with sample, but ranged among 4 to 8.

Once clear, 2 mL H_2O_2 was added to each tube. Samples were heated for 1 h and allowed to cool overnight. After samples were cooled, de-ionized water was added to nearly fill each tube. Samples were vortexed and allowed to cool to room temperature. Samples were brought to 75 mL with de-ionized water, capped, and inverted to mix. The solution was sub-sampled into 16

dram vials for analysis of plant N and P content on the Technicon autoanalyzer II (Technicon Industrial Systems, Tarrytown, NY). Plant N and P uptake (kg ha^{-1}) was calculated from N and P concentration using equation 3.7.

$$\text{N or P uptake (kg ha}^{-1}) = \frac{\text{dry weight (g m}^{-2}) \times \text{N or P concentration (}\mu\text{g g}^{-1})}{10^5} \qquad \text{(Eq. 3.7)}$$

The average N and P uptake values for each sequence were based on reported values in the literature and on specific crop yields. In order to relate average N and P uptake values to the sequences in this study, values were adjusted to the specific yields of pea from W-P, wheat from LGrM-W, wheat from M-W, and barley from W-B. For instance, average N uptake for barley with 3225 kg ha^{-1} grain yield is 58 kg N ha^{-1} in the grain (Wallace, 2001). The grain yield of barley from this study was 2010 kg ha^{-1} (U of S technical staff, 2012, unpublished). The average N uptake for barley with a yield of 2010 kg grain ha^{-1} instead of 3225 kg was calculated as follows, where *x* is the average N uptake of barley for this study:

$$\frac{58 \text{ kg N ha}^{-1} \text{ (average N uptake)}}{3225 \text{ kg ha}^{-1}} \times \frac{x}{2010 \text{ kg ha}^{-1} \text{ grain yield at Scott}} = 36 \text{ kg N ha}^{-1} \text{ (avg N uptake)} \qquad \text{(Eq. 3.8)}$$

3.4.6 Statistical analysis

All data was tested for normality with the Shapiro-Wilk test (Shapiro and Wilk, 1965) and no transformations were needed. Spore density, AMF colonization in the bioassay, harvest yield, and weed abundance results were analyzed using one-way analysis of variance (ANOVA).

Abundance of AMF biomarker 16:1ω5c, ALPase, and P_{mic} were analyzed using 2-way ANOVAs with time of sampling (pre-seeding or flowering) and treatment (cropping sequence where soil was sampled) as the main effects. Crop colonization by AMF in June, July, and August, dry shoot weights, and plant N and P concentrations and uptake were measured using 2-way ANOVAs with date and crop as the main effects. All statistics were analyzed in SPSS (IBM Corporation, Armonk, NY). Effects and interactions were considered significant at $p \leq 0.1$. Means were compared using Fisher's LSD test at $\alpha=0.1$. The significance level of $p \leq 0.1$ was chosen rather than $p \leq 0.05$ due to the high variability of many soil properties (Assefa et al., 2004). When applicable, correlation analyses were also performed with SPSS.

3.5 Results

3.5.1 Soil characterization and AMF colonization

Soil pH, NO_3-N, and PO_4-P levels from 0 to 15 cm soil samples are shown in Table 3.2. Soil NO_3-N was greatest in LGrM-W ($p=0.02$). Soil PO_4-P does not differ among sequences, but LGrM-W had a tendency for greater PO_4-P. The LGrM-W sequence had a tendency for lowest pH.

Spore density of AMF did not statistically differ among cropping sequences at pre-seeding ($p>0.1$), despite non-mycorrhizal mustard and the partial fallow period (LGrM), which would normally decrease the number of AMF spores due to the lack of host crops (Fig. 3.1). The W-B sequence had a tendency for lower SpD.

The biomarker 16:1ω5c was used to identify viable AMF spores (Olsson, 1999), and soil samples were analyzed at pre-seeding and flowering. Surprisingly, abundance was greatest following non-mycorrhizal mustard (M-W) at pre-seeding ($p=0.07$) (Fig. 3.2). There were no

statistical differences at flowering, but M-W once again had a tendency for higher biomarker abundance. Otherwise no differences were detected in any sequence on any date.

Percentage AMF colonization between cropping sequences did not vary (p>0.1) in June and July, including crops following the non-mycorrhizal crop (mustard) and partial fallow period (LGrM) (Fig. 3.3). In August, colonization by AMF in W-B was lowest (p=0.008) and LGrM-W was greatest (p=0.04). Colonization by AMF of wheat plants in the bioassay also did not differ among treatments (p>0.1) (Fig. 3.4), including soil from the partial fallow period (LGrM) and soil that was previously cropped with mustard (non-mycorrhizal).

Table 3.2. Characterization of pre-seeding soils sampled from different organic cropping sequences from Scott, SK 2012. Cropping sequences are wheat-pea (W-PP), lentil green manure-wheat (LGrM-W), mustard-wheat (M-W), and wheat-barley (W-B). Crops planted after pre-seeding sampling in 2012 are bold and italicized.

Cropping Sequence	pH	NO_3-N	PO_4-P
		kg ha^{-1}	
W-***PP***	6.2†	8.5b‡	17.0
LGrM-***W***	5.3	29.7a	18.4
M-***W***	6.0	9.6b	16.8
W-***B***	5.9	8.3b	14.1
$LSD_{0.1}$	**ns**	**3.06**	**ns**

†pH measured via 2:1 water:soil.
‡Soil samples were a composite of the 0-15 cm Ap horizon.
§Means followed by a different letter within columns are different according to $LSD_{0.1}$.

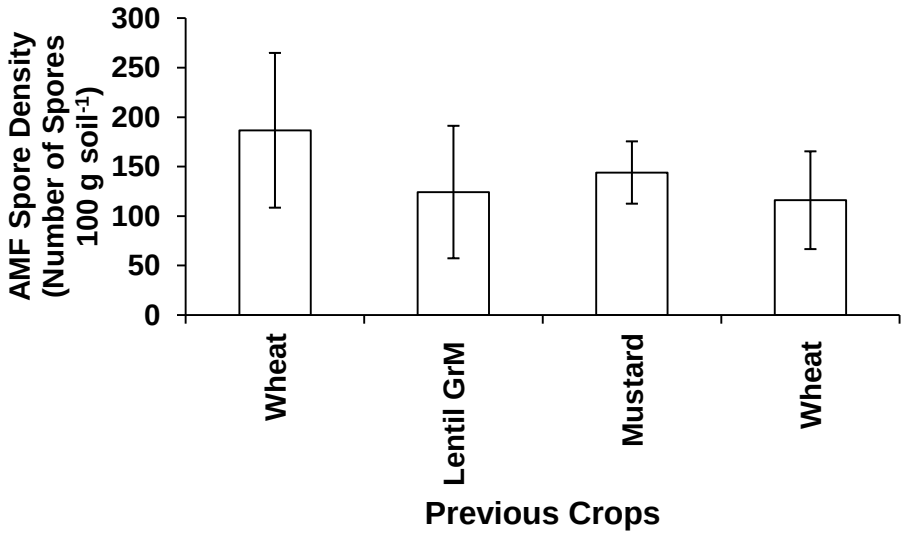

Fig. 3.1. Arbuscular mycorrhizal fungi (AMF) spore density at pre-seeding from soils sampled from different organic cropping sequences at Scott, SK in spring of 2012. The crops listed were planted and harvested in 2011. Lentil GrM denotes lentil green manure. Crops in 2011 included mycorrhizal crops (wheat, lentil) and non-mycorrhizal crops (mustard). There was also a partial fallow period (lentil GrM). Error bars are one SD of the mean. Means were not different according to $LSD_{0.1}$.

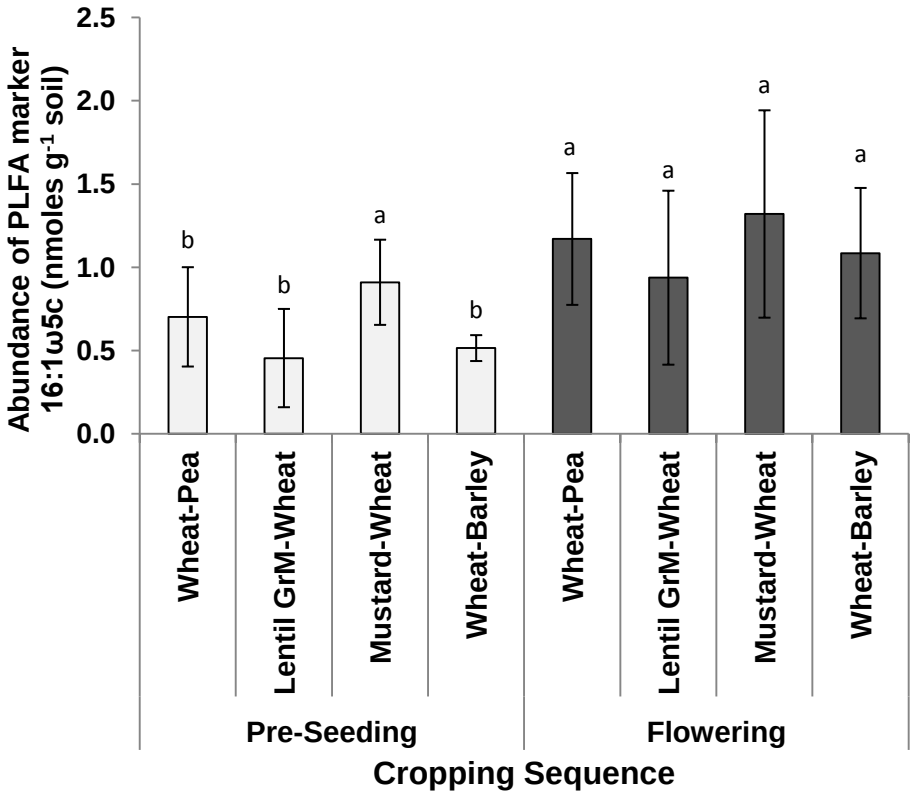

Fig. 3.2. Abundance of arbuscular mycorrhizal fungi (AMF) phospholipid fatty acid (PLFA) biomarker 16:1ω5c in soils sampled from different organic cropping sequences at pre-seeding and flowering from Scott, SK 2012. The first crop listed before the dash was planted in 2011, and the crop listed after the dash was planted in 2012. Lentil GrM denotes lentil green manure. Cropping sequences included crops that support uninterrupted mycorrhizal colonization (wheat-pea, wheat-barley), a partial fallow period (lentil green manure-wheat), and crops that interrupt mycorrhizal colonization (mustard-wheat). Different letters within sampling times indicate significant difference according to $LSD_{0.1}$.

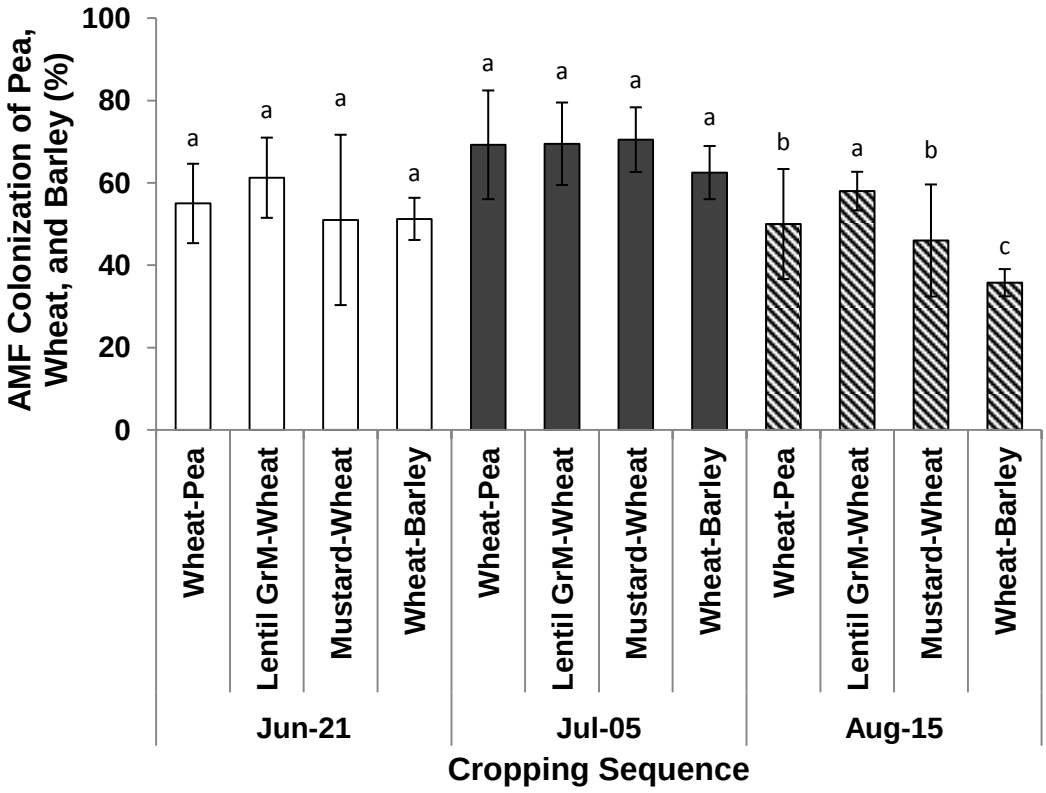

Fig. 3.3. Percentage arbuscular mycorrhizal fungi (AMF) colonization of crops grown in organic crop rotations at Scott, SK 2012. Crops were sampled from the field three times throughout the growing season. The first crop listed before the dash was planted in 2011, and the crop listed after the dash was planted in 2012. Lentil GrM denotes lentil green manure. Cropping sequences included crops that support uninterrupted mycorrhizal colonization (wheat-pea, wheat-barley), a partial fallow period (lentil green manure-wheat), and crops that interrupt mycorrhizal colonization (mustard-wheat). Error bars represent one SD of the mean. Different letters within sampling times indicate significant difference according to $LSD_{0.1}$.

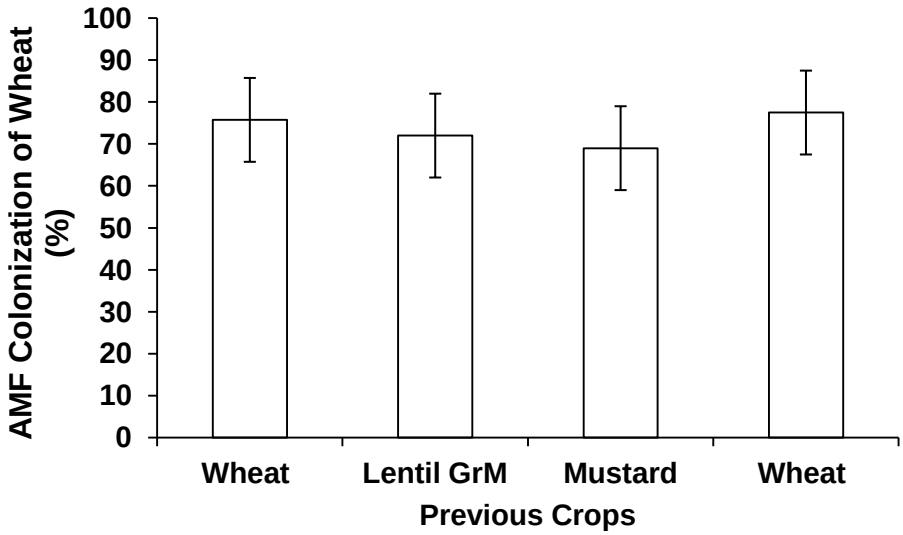

Fig. 3.4. Arbuscular mycorrhizal fungi (AMF) colonization of wheat in the bioassay. Composite soil samples were collected from organically managed plots at Scott, SK at pre-seeding in May 2012. All soils were planted with wheat, grown in the U of S campus greenhouse, and harvested after 8 wks to assess AMF colonization. Crops listed in the figure were planted in 2011. Lentil GrM denotes lentil green manure. Error bars represent one SD of the mean. Means were not significantly different according to $LSD_{0.1}$.

3.5.2 Soil microbial phosphorus

Alkaline phosphatase activity is reported as µg *p*-nitrophenyl (PNP) released g $soil^{-1}$ h^{-1}. Soil samples were taken for analysis at pre-seeding and flowering. The enzyme activity was greatest from W-B at pre-seeding (p=0.05), and W-B had a tendency for slightly higher PNP release at flowering (Fig. 3.5). Otherwise no differences were detected among cropping sequences at either sampling time.

Soil samples from pre-seeding and flowering were analyzed for P_{mic}. The P_{mic} of LGrM-W was greatest at pre-seeding following lentil plough down (p=0.009) and lowest at flowering

(p=0.1) (Fig. 3.6) compared to the other sequences. The W-B had a tendency for higher P_{mic} at pre-seeding than W-PP and M-W.

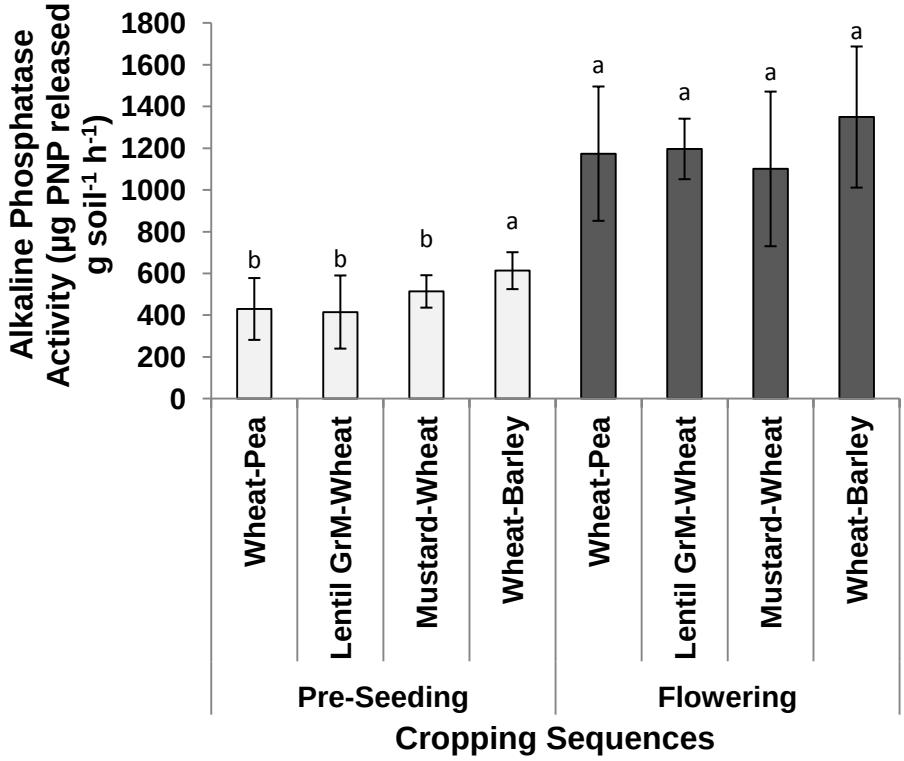

Fig. 3.5. Alkaline phosphatase (ALPase) enzyme activity in soils collected at pre-seeding and flowering from organic crop rotations in Scott, SK in 2012. The first crop listed before the dash was planted in 2011, and the crop listed after the dash was planted in 2012. Lentil GrM denotes lentil green manure. Cropping sequences included crops that support uninterrupted mycorrhizal colonization (wheat-pea, wheat-barley), a partial fallow period (lentil green manure-wheat), and crops that interrupt mycorrhizal colonization (mustard-wheat). Error bars represent one SD of the mean. Different letters within sampling times indicate significant difference according to $LSD_{0.1}$.

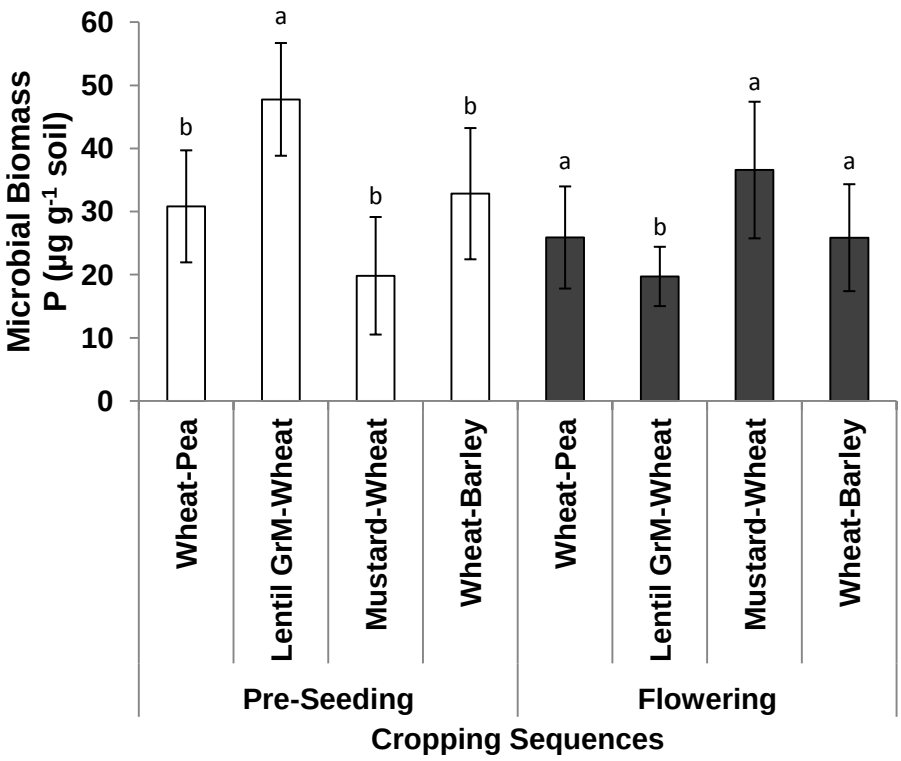

Fig. 3.6. Microbial biomass phosphorus (P) of soils collected from organic crop rotations in Scott, SK, 2012 at pre-seeding and flowering. The first crop listed before the dash was planted in 2011, and the crop listed after the dash was planted in 2012. Lentil GrM denotes lentil green manure, which was plowed down mid-season. Cropping sequences included crops that support uninterrupted mycorrhizal colonization (wheat-pea, wheat-barley), a partial fallow period (lentil green manure-wheat), and crops that interrupt mycorrhizal colonization (mustard-wheat).Errors bars represent one SD of the mean. Different letters within sampling times indicate significant difference according to $LSD_{0.1}$.

3.5.3 Plant phosphorus and nitrogen concentration and uptake

Plant samples were taken on June 21, July 5, and August 15. Plants were measured for N and P concentration and uptake of the above-ground biomass (Table 3.3, Table 3.4). Due to the varying N and P demands of each crop, it was only useful to compare concentrations to critical nutrient ranges (CNRs) and average uptake values reported in the literature. The focus was placed on the July 5 sampling date, as this date was the closest to flowering and the time of greatest crop growth activity (Malhi et al., 2006). Plant P concentration was within the CNR of all crops on July 5, while N concentration was below each crop's CNR (Table 3.3). Plant P and N uptake were average in all crops (Table 3.4). Nitrogen uptake of wheat in M-W was greatest compared to its average (81.8 kg N ha^{-1}) followed by wheat in LGrM-W (76.2 kg N ha^{-1}). Weed biomass (Table 3.5) was greatest in the W-PP and W-B sequences (1580 kg ha^{-1}, 910 kg ha^{-1}).

Table 3.3. Plant phosphorus (P) and nitrogen (N) concentration of crops from organic cropping sequences sampled on three dates from Scott, SK in 2012. Crop concentrations of P and N italicized and on July 5 are compared to their critical nutrient ranges (CNR) at flowering. Values in bold indicate sufficient concentrations of P or N. Cropping sequences are wheat-pea (W-PP), lentil green manure-wheat (LGrM-W), mustard-wheat (M-W), and wheat-barley (W-B). The italicized and bold letter in the cropping sequence denotes the crop sampled in 2012.

	Plant P concentration				CNR at flowering (P)	
Cropping Sequence	June 21	July 5	August 15	Harvest		
	----------	----------	----------	mg g^{-1} ----------	----------	
W-***PP***	2.35	***4.08a†***	0.80b	2.00a	Pea	***3.90 -6.70¶***
LGrM-***W***	2.35	***3.48b***	2.38a	1.29b	Wheat	***2.60-5.00♯***
M-***W***	2.93	***3.08b***	1.90a	1.79a	Wheat	***2.60-5.00***
W-***B***	2.40	***3.63b***	2.08a	2.12a	Barley	***2.00-5.00††***
$LSD_{0.1}$	**ns‡**	**0.58**	**0.52**	**0.50**	--	--
	Plant N concentration				CNR at flowering (N)	
Cropping Sequence	June 21	July 5	August 15	Harvest		
	----------	----------	----------	mg g^{-1} ----------	----------	
W-***PP***	25.3	*33.0a*	13.1a	23.8a	Pea	***45.0-51.0¶***
LGrM-***W***	23.7	*16.8b*	11.4a	8.20b	Wheat	***20.0-30.0♯***
M-***W***	24.6	*18.0b*	9.40b	10.9b	Wheat	***20.0-30.0***
W-***B***	23.7	*14.0b*	7.90b	8.90b	Barley	***40.0-50.0††***
$LSD_{0.1}$	**ns**	**4.43**	**3.59**	**3.80**	--	--

†Means followed by the same letter within a sampling date are not significantly different according to $LSD_{0.1}$.
‡ns indicates non-significance.
§Wheat-pea data not included from August due to pea termination.
¶ Source: P. Plancquaert, Institut Technique des Céréales et des Fourrages (ITCF), 1989
♯ Source: McKenzie, 1998. Government of Alberta.
†† Source: Southern Cooperative Series Bulletin #394, North Carolina Department of Agriculture, 2000

Table 3.4. Plant phosphorus (P) and nitrogen (N) uptake of crops from organic cropping sequences sampled on three dates from Scott, SK in 2012. Crop uptake of P and N on July 5 are italicized and compared to average uptake values. Values in bold indicate sufficient uptake. Average uptake values of N and P are adjusted according to harvest grain yield of each cropping sequence (Table 3.3). Cropping sequences are wheat-pea (W-PP), lentil green-manure wheat (LGrM-W), mustard-wheat (M-W), and wheat-barley (W-B). Letters italicized and in bold of cropping sequences denote crop sampled in 2012.

Cropping Sequences	Plant P uptake				Avg Plant P uptake‡ (at flowering)	
	---------- kg ha^{-1} ----------					
	June 21	July 5	August 15	Harvest		
W-***PP***	6.36b†	***11.2b***	2.05c	1.72b	Pea	***7.10***
LGrM-***W***	14.5a	***18.8a***	14.3a	3.50a	Wheat	***15.0§***
M-***W***	15.4a	***16.5a***	9.14b	4.39a	Wheat	***14.0***
W-***B***	5.76b	***7.00c***	4.44c	2.27b	Barley	***6.50***
$LSD_{0.1}$	**4.70**	**3.89**	**2.54**	**1.46**	**--**	**--**
Cropping Sequences	Plant N uptake				Avg Plant N uptake‡ (at flowering)	
	---------- kg ha^{-1} ----------					
	June 21	July 5	August 15	Harvest		
W-***PP***	69.1b	***87.4a***	34.5a	20.5a	Pea	***32.0***
LGrM-***W***	93.2a	***137a***	65.8a	21.7a	Wheat	***61.0***
M-***W***	99.9a	***138a***	49.5b	26.8a	Wheat	***57.0***
W-***B***	28.9b	***54.8b***	16.6c	9.48b	Barley	***36.0***
$LSD_{0.1}$	**33.7**	**46.2**	**17.0**	**6.68**	--	--

†Means followed by the same letter within sampling dates are not significantly different according to $LSD_{0.1}$.
‡Source: Wallace, 2001.

Table 3.5. Weed biomass from plots sampled in 2012 in Scott, SK . Cropping sequences are wheat-pea (W-PP), lentil green-manure wheat (LGrM-W), mustard-wheat (M-W), and wheat-barley (W-B). Letters italicized and in bold of cropping sequences denote crop sampled in 2012.

	kg ha^{-1}
W-***PP***	1580a†
LGrM-***W***	113b
M-***W***	105b
W-***B***	910a
$LSD_{0.1}$	**339**

†Means followed by the same letter within sampling dates are not significantly different according to $LSD_{0.1}$.

3.6 Discussion

Activity of AMF

The lack of a difference in AMF SpD at pre-seeding indicates that each cropping sequence had a roughly equivalent number of spores stored from the previous growing season. Measurement of biomarker 16:1ω5c at pre-seeding revealed which sequence had the highest number of viable AMF spores. The wet-sieving technique used to extract AMF spores from soil does not differentiate between viable and non-viable spores (An et al., 1990) and the AMF biomarker 16:1ω5c represents the viable portion of AMF biomass in soil (Olsson, 1999). Systems with high AMF SpD and low 16:1ω5c abundance have a large but dormant population of AMF spores (An et al., 1990; Olsson et al., 1999). The increase in 16:1ω5c abundance of all cropping sequences between pre-seeding and flowering reflected the presence of growing crops and the increase in AMF activity due to host crop colonization. The even colonization of wheat in the bioassay reflects the similar number of viable AMF spores in all plots despite non-mycorrhizal crops or fallow periods in rotation.

Many studies have found greater AMF SpD under diverse cropping rotations, such as those in the AAFC-ACS experiment, than under monocultures (Smith, 1980; An et al., 1990). A

3-yr experiment examining the effects of various crop rotations with soybean (*Glycine max* L.), tall fescue (*Festuca arundinacea*), sorghum (*Sorghum bicolor*), and corn on AMF SpD found the lowest AMF SpD in continuous soybean plots and the highest AMF SpD in cropping rotations that contained all three crops (An et al., 1990). Overall AMF SpD was three-fold greater in plots under diverse crop rotations than in the continuous soybean plots (An et al., 1990).

The lack of variation in ALPase activity is also related to AMF growth. One study using trypan blue staining noted that a sharp increase in ALPase activity of AMF mycelium (4-45%) in the roots of pea coincided with plant growth response to infection by AMF, seen in significantly increased dry shoot weights of pea (Tisserant et al., 1993). The roots of the non-mycorrhizal control plants (pea cv. Frisson, myc^- nod^- mutant) did not stain for ALPase. They concluded that activity of the ALPase enzyme is closely linked to AMF metabolism, and represents an important component of the symbiosis (Tisserant et al., 1993). Further studies have seen similar increases of ALPase activity in AMF mycelium of plant roots directly before plant growth responses to AMF infection (Gianinazzi-Pearson and Gianinazzi, 1978; Guillemin et al., 1995; Jabaji-Hare et al., 1990). Alkaline phosphatase activity was measured in soil rather than plant roots for this study, but ALPase activity originates entirely from microbial (i.e. fungal) activity (Hebrien and Neal, 1990). Thus the ubiquitous nature of ALPase in soil at flowering likely originates partially from AMF mycelium in soil.

Colonization by AMF in June and July did not differ among cropping sequences. The lack of a difference among AMF SpD at pre-seeding, 16:1ω5c abundance at flowering, and ALPase activity at flowering is reflected in the even colonization of all crops in June and July, and indicates that AMF spores and DNA are preserved in all measured soil despite previous crop type.

Colonization of wheat in the bioassay also did not differ between treatments. The wheat plants in the bioassay were harvested at flowering (8 wks), and their ubiquitous colonization by AMF mirrors the unusual colonization patterns of AMF seen in the field soil. The bioassay confirmed the unanimous presence of viable AMF spores in the soil of all cropping sequences. Despite previous studies to the contrary (Gavito and Miller, 1998; Karasawa et al, 2002), mustard (non-mycorrhizal) from the M-W sequence and the partial fallow period of LGrM-W did not decrease AMF SpD in soil or depress AMF colonization of the wheat following them.

The colonization rates seen here (36% to 71%) are comparable to those seen in other organic studies. Entz et al. (2004) found 59% to 81% colonization of organic flax in P deficient soil (<10 kg P ha^{-1}) 40 d after planting, Mäder et al. (2000) found an average of 55% colonization of organic winter wheat from 6 sampling times over 2 yrs in P deficient soil (<20 kg P ha^{-1}), Ryan et al. (2005) found 24 to 66% colonization of wheat in P deficient soil (<20 kg P ha^{-1}) and Baird et al. (2010) found 75% to 80% average colonization of organic field pea in low-P soil (<20 kg P ha^{-1}) sampled at 10 d intervals over the growing season.

Additionally all crops are colonized to a similar degree in June and July despite differing root systems. Crops like barley and wheat have fine and extensive root systems and are generally less dependent on the AMF symbiosis than crops with coarse roots like pea (Plenchette et al., 1983; Ryan and Angus, 2003; Ryan et al., 2005). Fine roots increase the area of soil exploration, and thereby the ability to reach immobile soil P (Plenchette et al., 1983). Thus it was expected that pea would be colonized to a greater degree than wheat or barley.

Arbuscular mycorrhizal fungi are negatively affected by soluble P inputs (Ryan and Graham, 2002; Beauregard et al., 2010) and high soil P content (>20 kg P ha^{-1}) (Allen et al., 1981; Douds and Schenk, 1990; Vivekanandan and Fixen, 1991). A study in SK found that as

additions of inorganic P-fertilizers increased, AMF colonization of alfalfa decreased, but abundance of the AMF biomarker 16:1ω5c in the soil was unaffected (Beauregard et al., 2010). This indicates that as more soil P is made available or unavailable to plants, AMF reacts accordingly. Therefore, it is possible that AMF responded to the lack of P inputs, low plant-available P levels, and diverse crop rotations in this organic system and colonized each crop equally in June and July. In conventional systems, the addition of inorganic P fertilizers would decrease effectiveness of AMF (Asimi et al., 1981; Douds and Schenk, 1990; Beauregard et al., 2010).

The unusual colonization patterns of M-W and LGrM-W can be partially explained by crop rotation. Surprisingly, non-mycorrhizal mustard and the partial fallow period (LGrM) did not decrease AMF SpD or 16:1ω5c abundance and did not delay AMF colonization of wheat compared to the mycorrhizal sequences (W-PP and W-B). In fact, M-W had the greatest abundance of the AMF biomarker 16:1ω5c at pre-seeding, and LGrM-W has the highest percentage of AMF colonization in August. In conventional systems, non-mycorrhizal crops and partial fallow periods in rotation generally decrease the activity of AMF in soil and decrease spore counts, as there is no host plant for the fungus to colonize (Gavito and Miller, 1998; Karasawa et al., 2002).

Previous studies have noted increases of AMF species richness, biomass, and productivity following N enrichment of soils with low levels of plant-available P (Eorn et al., 1999; Egerton-Warburton et al., 2007). One study examining the response of AMF communities to N fertilization across low- and high-P grassland soils throughout the United States noted that N fertilization of P limited soils (<20 kg P ha^{-1}) showed significant increases in AMF crop colonization, species richness, and species diversity (Egerton-Warburton et al., 2007). They did

not report this effect in soils where P was not limiting. The study concluded that N enrichment of P-limited soils widens the soil N:P ratio, rendering soil P less available for plants and AMF more active (Egerton-Warburton et al., 2007). Other studies have noted increases of AMF SpD and crop colonization after soil N enrichment of P-limited soil (Anderson et al., 1994; Eorn et al., 1999).

Although there were no fertilizer N inputs in this system, there was soil N enrichment from crop rotation. Alfalfa was grown in the M-W plots for three years before mustard was planted in 2011. Alfalfa fixes N_2 (Watson et al., 2002a), and is known to provide extended availability of N to crops (Hoyt, 1990; Kelner et al., 1997). A study in MB found that a 3-year rather than a 1-year period of alfalfa added 137 versus 84 kg total N ha^{-1} to soil, respectively (Kelner et al., 1997). They concluded that the amount of soil N for subsequent crops increased with the length of the alfalfa stand (Kelner et al., 1997). A further study conducted in northern AB found that wheat grown for nine consecutive years following alfalfa stands (2 to 6 yrs) had 81% greater yield on average than wheat monoculture (Hoyt, 1990). They postulated that the deep rooting systems of alfalfa were extracting N from the subsoil and making it available for wheat over the long term (Hoyt, 1990). Thus, there are likely residual N effects from the 3-yr alfalfa stand that was terminated two years prior (2010) to wheat (2012).

In the LGrM-W sequence, the plough down of lentil added N to the soil. Many young legumes, such as the LGrM ploughed down here, have narrow C:N ratios that readily decompose in soil and add N (Foster, 1990; Biederbeck and Bouman, 1994; Biederbeck et al., 1998). One study found that soil N deposition was on average 56% greater from the legume green manures chickling vetch (*Lathyrus sativus* L.), black lentil (*Lens culinaris* Medikus), Tangier flatpea (*Lathyrus tingitanus* L.), and feedpea than continuous wheat (Biederbeck et al., 1998). The

effects of the LGrM plough-down are apparent in the soil N level of this sequence at pre-seeding, which is greater than all others. The positive effects from soil N enrichment on AMF colonization are also seen in LGrM-W in August, where colonization of wheat is greater than the other sequences.

Soil N enrichment from the alfalfa stand and LGrM likely widened the soil inorganic N:P ratio, preserved AMF spores, and increased AMF activity (Egerton-Warburton et al., 2007). The inorganic soil N:P ratios in LGrM-W (1.6) and M-W (0.57) are quite narrow, but there is no minimum value of soil N:P required to see an increase in AMF activity. Any addition of N to P limited soils can enhance AMF activity (Anderson et al., 1994; Eorn et al., 1999; Egerton-Warburton et al., 2007). What is necessary is the relative availability of soil inorganic N to soil inorganic P before and after soil N enrichment. Once soil inorganic N is added, in any amount, it widens the soil N:P ratio, and no matter how small of an increase, P is rendered less available and AMF more effective.

As mentioned, however, non-mycorrhizal crops and partial fallow periods are widely known to delay colonization of following crops in conventional systems commonly utilizing N fertilizers (Gavito and Miller, 1998). Soil N enrichment was therefore only part of the explanation for these trends. The combination of soil N enrichment, low soil P_i, and no soluble P inputs likely created a scenario unique to this organic farming system. The activity of AMF, already heightened by low levels of soil P_i, increased as N:P widened due to soil N deposition from LGrM and alfalfa. The resulting unavailability of P was more influential than non-mycorrhizal crops, partial fallow periods, or differing root systems in conventionally managed soil.

Unlike wheat and pea, AMF colonization of barley decreased between July and August (63% to 36%). This decrease in AMF colonization may be related to a lack of soil N enrichment, although this was not directly measured. Unlike the other sequences, W-B had no legumes in rotation within the last 3 yrs before sampling, and thus no recent N input from crop rotation. Conditions that normally influence AMF colonization (i.e. non-host periods and crop dependency on AMF) were altered in this system.

Spore density and DNA of AMF and colonization by AMF may have also persisted due to the presence of mycorrhizal weed species. Weed diversity in organic systems is typically greater than in conventional systems (Chen et al., 2005), and the mycorrhizal status of weeds can affect AMF crop colonization. A conventional farming study of maize monocultures found that yields of maize were negatively impacted with the removal of mycorrhizal weeds (Feldmann and Boyle, 1999). Another study in an organic system found that the presence of non-mycorrhizal weeds either reduced or had no effect on the colonization of pea in an organic system (Fontanela et al., 1999). The lack of herbicides used on the research plots could have preserved AMF spores due to the presence of host crops in all rotations, and in turn indirectly promoted AMF colonization through mycorrhizal weed growth. However, the mycorrhizal status of the weeds was not specified and weed biomass varied greatly between sequences. For instance, weed biomass was lower (105 kg ha^{-1}) in the M-W sequence and greater (1580 kg ha^{-1}) in the W-PP sequence. Alfalfa, grown from 2008-2010 in the M-W plot, is known to smother weeds (Abdul-Rahman and Habib, 1989; Chung and Miller, 1995; Miller, 1996), while pea is often a poor competitor with weeds (Liebman and Dyck, 1993; van Kessel and Hartley, 2000; Hauggaard-Nielsen et al., 2001). Thus the influence from weeds is not constant within the sequences and is somewhat related to crop type. Furthermore if mycorrhizal weeds had a significant effect on

AMF colonization, this does not explain why the colonization of W-B is reduced in August, as this sequence had the second highest weed biomass. Without measuring the root systems of individual weed species it is difficult to know how much of an influence they actually had on the crops.

Crop P and N

Crop rotation also influenced crop P and N concentrations and uptake. In order to normalize all data, crops were compared to their CNRs and average uptake levels at flowering. A study by Malhi et al. (2006) measured the P and N nutrient uptake patterns of wheat and barley in Melfort, SK over two separate years. They found that in general, uptake of both nutrients followed a steep curve that peaked 61-75 days after emergence (DAE), or around flowering of the plants. Following this period, uptake leveled off and either stabilized or decreased slightly until harvest. The focus of this discussion largely concerns these factors from the July 5 sampling date, as it is the closest to flowering and the growth stage with the greatest crop uptake of both P and N.

All plant P concentrations and uptake fell within their CNRs and average uptake levels at flowering. Uptake of P generally followed patterns reported by Malhi et al. (2006), with greatest uptake at flowering followed by stabilization and a slight decrease until harvest. The sufficient P concentration and uptake of all crops may indicate AMF activity, as the symbiosis is known to enhance plant P uptake (Bolan, 1991; Smith and Read, 1997).

Sufficient P-uptake by all crops may also indicate mineralization of P in soil. The activity of the ALPase enzyme induces chemical transformations of P_o to P_i (Kremer and Li, 2003), and is quite high in this study (451-1349 µg PNP released g soil^{-1} h^{-1}) compared to the literature. Reported values in conventional systems can range 300-1080 µg PNP released g soil^{-1} h^{-1} (Hu et

al., 2009; Okur et al., 2009; Hu et al., 2010) and 350-1200 µg PNP released g soil^{-1} h^{-1} in organic systems throughout the growing season (Lalande et al., 1998; Kremer and Li, 2003; Okur et al., 2009). The activity of ALPase originates from bacteria and fungi but not from plants (Hebrien and Neal, 1990), and ALPase activity of microorganisms is known to increase in soils with low P_i content (Calleja and D'Auzac, 1983; Li et al., 1997). Thus the elevated activity in this system may result from low soil P_i levels, and indicates an active microbial population that is rapidly mineralizing soil P. Phosphorus uptake by all crops was likely influenced by AMF colonization as well as ALPase activity in this organically-managed soil.

High soil P_o content can also result in elevated ALPase activity (Sharpley, 1985; Tarafdar and Jungk, 1987). One study noted that the depletion of soil P_o from the rhizospheres of onion (*Allium cepa*), mustard, wheat, and clover (*Trifolium alexandrinum*) was positively correlated to ALPase activity in a greenhouse study (Tarafdar and Jungk, 1987). Soil tests used solely for the measurement of soil P_i do not account for soil P_o, and thus do not represent the mineralization potential of soil phosphorus. Soil P_o was not measured for this study, therefore it is possible that soil P_o levels undetected by the soil test are resulting in enhanced ALPase activity, which resulted in elevated rates of P_o mineralization and greater levels of soil P_i for uptake by all crops.

Wheat from the LGrM-W and M-W sequences showed the greatest uptake of P. The greater P uptake of wheat in LGrM-W is most likely due to the green manure in sequence. Decomposition of legume green manures releases labile P into soil and increases P uptake of following crops (Cavigelli and Thien, 2003). Decomposition also releases organic acids that dissolve soil mineral P for increased crop uptake (Sharpley and Smith, 1989).

The 3-yr alfalfa stand planted prior to mustard in M-W likely contributed to P nutrition of both mustard and wheat. Alfalfa is also a legume, and aside from the decomposition of their

plant residues, legumes in crop rotation can increase P uptake of following non-legumes (MacLeod, 1999). This is due to increased colonization of roots by AMF and increased crop root growth (MacLeod, 1999). Furthermore roots of legumes can acidify soil and solubilize phosphate from calcium phosphate ions often found in Prairie soils (MacLeod, 1999; Malhi et al., 2002). The acidification effect is also noted in the pH of this sequence, which tended to be lower than all the others. As mentioned previously, the nutritional benefits from alfalfa for following crops extend for years beyond its termination (Hoyt, 1990). Thus elevated P uptake of wheat in M-W likely resulted from the alfalfa stand.

Plant N concentrations are deficient in all crops and uptake of N is among average levels in all sequences, and largely follows normal patterns reported by Malhi et al. (2006). The discrepancy between N uptake and concentrations by all crops in July may be a result of the translocation of N from plant tissue to seeds, although the passage of N within sampled crops was not traced. One study found that the reallocation of N to seeds before and during flowering of *Arabidopsis thaliana* was two-fold greater in a low N versus high N environment (Masclaux-Daubresse and Chardon, 2011). The crops in July were sampled during heading (wheat and barley) and flowering (pea) stages, therefore the low N concentrations in the plant tissue may indicate reallocation of N to seeds. Again, N movement within crops was not directly measured.

Soil N enrichment also influenced N uptake by wheat in LGrM-W and M-W. All crops had average N uptake, but N uptake of wheat in LGrM-W and M-W had the greatest uptake compared to their averages. Alfalfa stands and plough down of LGrM are often followed by soil N deposition (Foster, 1990; Biederbeck et al., 1998). One study conducted in the Canadian Prairies comparing N deposition from continuous wheat and wheat following four lentil green manures (feedpea, chickling vetch (*Lathyrus sativus* L.), black lentil (*lens culinaris* Medikus),

and Tangier flatpea (*Lathyrus tingitanus* L.)) noted that N enrichment was on average 56% greater than from continuous wheat (Biederbeck et al., 1998). Soil N deposition for this study was not directly measured, but the N effects from LGrM are apparent in the elevated levels of soil inorganic N in LGrM-W at pre-seeding. Additionally residual effects of alfalfa prior to M-W are likely contributing to N uptake of wheat in M-W (Hoyt, 1990).

Additionally AMF may have influenced plant uptake of N (Smith and Read, 1997; Subramanian and Charest, 1998). One study measuring N uptake of maize after AMF inoculation versus uninoculated maize found significantly greater N uptake in inoculated maize (Subramanian and Charest, 1998). They concluded that inorganic N was transported through AMF hyphae, which triggered increased activity of enzymes important for plant N assimilation (Subramanian and Charest, 1998). Nitrogen contents of roots and AMF hyphae was not measured in this study, however, so the actual influence of AMF on plant N uptake is not known.

Activity of ALPase

Aside from greater ALPase activity in W-B at pre-seeding, activity of the enzyme did not vary in this study. Unlike acid phosphatase, the activity of which originates from both plants and microbes (Tarafdar and Marschner, 1994; George et al., 2002), the activity of ALPase originates from bacteria and fungi but not from plants (Hebrien and Neal, 1990; Kramer and Green, 2002). The greater (p=0.05) activity of ALPase in W-B at pre-seeding is therefore not related to crop activity, but to the microbial activity in the soil. One study postulated that an increase of ALPase activity in soil cropped with maize (cv. Borias) was related to stimulation of SMB (George et al., 2002). A further study observed a significant correlation (r=0.73) between ALPase and SMB in an organic system (Okur et al., 2009). There was no significant correlation (r=0.20, p=0.46) between P_{mic} and ALPase in this study, however. The mechanisms for this increase are unclear.

Rather than increased activity of SMB, ALPase in W-B at pre-seeding may be related to AMF activity. The W-B sequence had a tendency for the lowest soil P_i at pre-seeding. One study adding different concentrations of inorganic phosphate (KH_2PO_4) to greenhouse soil (0.1 m*M* and 1.0 m*M*) found that AMF SpD, AMF colonization of wheat, and ALPase enzyme activity decreased as KH_2PO_4 concentrations increased (Balaz and Vostaka, 1997). However, W-B has a tendency for lower AMF SpD and 16:1ω5c abundance, indicating slightly lower levels of AMF activity in this sequence than the others. Thus the increase in ALPase activity is only partially related to AMF activity, and perhaps more related to microbial response to low soil P_i (Calleja and D'Auzac, 1983; Li et al., 1997). Its activity may also be related to the presence of high levels of indigenous P_o in soil, which is known to enhance ALPase enzymatic activity (Joner and Jakobsen, 1995b). Soil P_o was not measured, however, so its concentration in soil of W-B is not known.

Microbial Biomass P

Microbial biomass P (P_{mic}) size differed between cropping sequences at pre-seeding and at flowering. In agricultural systems, the long-term control over SMB is organic matter (Kaschuk et al., 2010), and the activity of P_{mic} is related to the quantity and quality of C substrate added to soil (Oberson et al., 1996; Ofori-Frimpong and Rowell, 1999; Schomberg and Steiner, 1999). One study observed that the immobilization of P by P_{mic} was proportional to the amount of soluble C added to the soil, and that soil P immobilization and mineralization is related to substrate degradability (Buenemann, 2003). The significantly greater P_{mic} (p=0.009) in LGrM-W at pre-seeding is most likely due to the mid-season plough down of the LGrM, which added a soluble C source for P_{mic}. The relatively young age of the legumes meant they were susceptible

to mineralization of C and P, which ultimately lead to tie-up of P within P_{mic} (Biederbeck et al., 1998; Buenemann, 2003).

At flowering, P_{mic} of LGrM-W was lower than all other sequences (p=0.1). This was most likely due to the exhaustion of substrate. The rapid mineralization rates of nutrients from legume GrM plough-down are usually observed only over the short term (Foster, 1990; Biederbeck et al., 1998; Lupwayi et al., 2006b). One 6-yr study found that decomposition of the green manures black lentil, Tangier flatpea, chickling vetch, and feedpea did not contribute greater C to soil than continuous wheat rotations (Biederbeck et al., 1998). Addition and removal of C sources (i.e. LGrM) within these cropping sequences correlated with P_{mic} size.

Conclusions

Conditions specific to this organic system influenced colonization of wheat, pea, and barley by AMF. The combination of low soil P_i levels, no inputs of soluble P, and soil N enrichment from crop rotation were more influential for AMF colonization than non-mycorrhizal crops or partial fallow periods in rotation. Furthermore crops were colonized evenly despite their differing root systems. The presence of AMF host weeds in the sequences likely influenced AMF colonization as well.

Concentrations and uptake of P are sufficient in all crops at flowering. This was likely a result of AMF, but was also influenced by P mobilization from legumes in crop rotation and microbial P turnover by ALPase. Soil N enrichment from crop rotation also influenced crop N uptake, which was greatest in LGrM-W and M-W due to LGrM and alfalfa in rotation. Crop rotation also influenced the activity and size of P_{mic}, which was increased following LGrM plough down. Microbial biomass P of cropping sequences that did not involve additions of C did not change between pre-seeding or flowering.

In summary, the study showed the vital importance of proper crop rotation in organic systems for soil and plant nutrition, and possibly, for the preservation of AMF.

4. SOIL PHOSPHORUS DYNAMICS FOLLOWING ADDITIONS OF BONE MEAL, HYDROXYAPATITE, AND COMPOST IN AN ORGANICALLY-MANAGED GREENHOUSE STUDY

4.1 Preface

Soil P is often unavailable to plants on SK organic farms, and the restriction on inputs in organic farming limits the soil P amendments that farmers may add to their systems. This experiment analyzes the effects of hydroxyapatite (HAP), bone meal (BM), and compost on soil P dynamics, plant P and N nutrition, and activity of AMF. While the field study measured AMF colonization, soil P dynamics, and plant N and P nutrition without the addition of any amendments, this study examines these processes after the addition of soil amendments approved for organic systems.

4.2 Abstract

Organically-managed lands in SK are often deficient in plant-available P. Organic farming prohibits the use of synthetic fertilizers for soil P fertilization, but some materials are approved for use, including bone meal (BM), rock phosphate (RP), and composted livestock manure. Each has the potential to increase plant-available P through P-dissolution in soil. Arbuscular mycorrhizal fungi (AMF) also increase plant P supply to host plants through the extension of root systems. The addition of slow-release P fertilizers such as BM, RP, and composted manure has varying effects on AMF colonization including an increase, decrease, and no change in colonization. The addition of slow-release P fertilizers to soil also directly affects soil microbial processes, as soil microbial biomass (SMB) and activity of the alkaline phosphatase (ALPase) enzyme are very sensitive to land management changes.

The objectives of this greenhouse study were to measure the effects of applications of BM, hydroxyapatite (HAP) and sheep manure compost (ShMC) alone and in combination on AMF colonization of wheat (*Triticum aestivum* L.), alkaline phosphatase (ALPase) activity, microbial biomass P (P_{mic}), and plant P and N nutrition. Colonization by AMF and ALPase were more related to one another than treatment. Treatments with ShMC increased P concentrations of wheat on average compared to the control (1.99 mg g^{-1} versus 1.43 mg g^{-1}) and plant N concentrations did not differ between treatments. Uptake of P was 1.26 mg pot^{-1} in ShMC treatments versus 0.71 mg pot^{-1} in treatments without ShMC, and uptake of N by wheat did not differ between treatments. Activity and size of P_{mic} was not consistently related to treatment. Bone meal and HAP did not affect N and P uptake of wheat. Overall, ShMC was an immediate source of P for wheat while BM and HAP were not.

4.3 Introduction

Organically-managed land in the Canadian Prairies is generally deficient in plant-available phosphorus (P) due to binding of P with various cations (Malhi et al., 2002). While conventional farmers use inorganic P fertilizers for plant P nutrition, the use of P inputs is largely restricted in organic systems (Canadian General Standards Board, 2011). Aside from crop rotation, there are limited options available for soil P fertility on organic farms.

Arbuscular mycorrhizal fungi (AMF) are useful to organic farmers for increasing plant-available P. They occur naturally in soils, and form a symbiotic relationship with approximately 80% of terrestrial plants (Zhu et al., 2007). The fungi colonize the roots of host plants and increase access to immobile soil P in exchange for carbon (C) (Bolan, 1991; Smith and Read, 1997). Crop colonization by AMF is generally greater in organic than conventional systems (Mäder et al., 2000; Oehl et al., 2002; Entz et al., 2004), due partially to the decrease in AMF colonization occurring after inputs of P in conventional farming (Asimi et al., 1980; de Miranda et al., 1989; Hinsinger, 2001). One study in SK found that crop colonization by AMF decreased as inputs of inorganic P fertilizer to soil increased, but that the abundance of AMF DNA in soil was unaffected (Beauregard et al., 2010). This suggests that AMF are not harmed by P-inputs, but that colonization adjusts according to host crop P needs.

Some P-inputs are approved for use in organic farming, including bone meal (BM), rock phosphate (RP), and composted manure (Canadian General Standards Board, 2011). At the time of writing, the use of soil P amendments is not common among organic farmers in SK (Knight et al., 2010a). Out of 39 surveyed organic producers in SK, only three applied RP to their fields, and only six applied some form of composted livestock manure (Knight et al., 2010a).

The use of BM is rare in any organic farming system (Nelson and Janke, 2007). Despite past fears surrounding the possibility of mad cow disease transference from BM (Kamphues,

2002), it is a potential source of N and P for crops (Novelo et al., 1998; Jeng et al., 2004 and 2006; Mondini et al., 2008). The availability of P from BM in soil depends on the dissolution of the $Ca_5(PO_4)_3OH$ (hydroxyapatite, HAP) component, which is heavily dependent on pH. The dissolution reaction requires H^+ ions, meaning BM will more readily dissolve in soils with pH<6 (Bekele and Hofner, 1993; Surendra et al., 1993).

The application of BM to soils can increase plant N and P uptake (Jeng et al., 2004 and 2006). One study found that when meat and BM were applied to both acidic and alkaline soils in a pot experiment according to the N demands of rye grass (*Lolium perenne*) and spring barley (*Hordeum vulgare*), P demands were more than sufficiently met (Jeng et al., 2006). Researchers also noted an increase in yield of both crops as applications of BM increased (Jeng et al., 2006).

Bone meal also contains organic residue, and its application has resulted in increases in SMB (Novelo et al., 1998; Mondini et al., 2008). A greenhouse study found a two-fold increase in SMB and ALPase activity 2 d after BM application to both acidic and alkaline soils (Mondini et al., 2008). Furthermore activity of SMB and ALPase were sustained throughout the incubation period (Mondini et al., 2008). Another study found that SMB increased after BM application to coffee plantation soils (Novelo et al., 1998). The results of these studies indicate that BM can positively impact plant P nutrition and SMB and can be a readily-available source of P in soils of varying pH.

Rock phosphate (RP) is sometimes used on organic farms as fertilizer due to its P content, although its performance is erratic (Takeda, 2004; Shirtliffe and Knight, 2006). The main component of RP is HAP. Dissolution of P from the material occurs more readily in soils with pH<6 (Bekele and Hofner, 1993). Many of the cultivated soils in SK have pH>6 due to calcium carbonate in the parent material (Malhi et al., 2002), and thus RP has shown limited

potential within the province. One study in SK found that RP did not affect plant P concentration, plant biomass, plant P-uptake, or yields of several crops unless it was applied in combination with fungal inoculants (Takeda, 2004). Another study on several organically-managed fields in SK found that although RP application to soil increased yields of wheat (*Triticum aestivum* L.) and pea (*Pisum sativum* L.), the increase was not enough for RP to be economically viable (Shirtliffe and Knight, 2006).Therefore RP may be more effective when applied in combination with other amendments.

Livestock manure is a valuable resource for organic farmers, as many of the nutrients consumed by livestock are preserved in their manure (Adler and Sikora, 2003). Manure applications can increase crop yields, plant biomass, and nutrient concentrations in plant tissue (Smith et al., 1997; Delschen, 1999; Stamatiadis et al., 1999; Brandt et al., 2007). The availability of nutrients from manure relies on time of application, what is fed to the livestock, manure storage, and the type of animal (Schoenau et al., 2010). It also depends on whether nutrients are present in plant-available forms. When N and P within manure are in organic forms, they must become plant available over time (Schoenau et al., 2010). For example, the N in cattle manure is largely organic N, meaning it becomes more available after the first year of application (Qian and Schoenau, 2002).

The availability of N and P from manure also relies on their balance with C, which governs mineralization and immobilization. Mineralization is the process that renders N and P plant-available, and immobilization occurs when microbes assimilate N and P, rendering them unavailable to plants (Tisdale and Nelson, 1975). Eventually microbes will release N and P back into soil upon their death. Generally, when C:N<20 and C:P<200, net mineralization of N and P occurs, respectively, while net immobilization occurs if C:N>30 and C:P>300 (Tisdale and

Nelson, 1975). When ratios are between these values, neither process occurs more often than the other.

Organic farmers generally use composted rather than raw manure, as nutrients from composted manure are more stable and considered less harmful to the environment (Seiter and Horwath, 2004; Walz, 1999). Additions of composted manure to crops are beneficial in the short- and long-term (Smith et al., 1997; Delschen, 1999; Stamatiadis et al., 1999; Carpenter-Boggs et al., 2000; Leifeld et al., 2002), and can increase SMB size and activity (Mäder et al., 2002; Fliessbach et al., 2007).

During the composting process, up to 50% of total N can be converted to organic forms (Adler and Sikora, 2003), while P is preserved (Eghball and Power, 1999; Eghball et al., 2002). Generally the C:N:P ratio of soil organic matter (SOM) is 100:10:1, and when N or P is limiting, it induces mineralization of the other (Tisdale and Nelson, 1975). The loss of N in composted manure narrows the N:P ratio, causing mineralization of P (Adler and Sikora, 2003). One study found that composted manure with less than 0.01% plant-available P was still an ample source of P for plant uptake (Sikora and Enkiri, 2005). However the heightened mineralization of P from composted manure means that applications to meet plant N requirements can result in loading of soil P, which may lead to P run-off (Douds et al., 1997; Mikkelsen, 2000).

Some researchers have observed increased effectiveness of compost when combined with other amendments (Nishanth and Biswas, 2008; González et al., 2010; Verma et al., 2013). One study found higher yields and greater plant N and P uptake by beet (*Beta vulgaris*) after combined applications of vermicompost and BM to soil versus vermicompost applied alone (González et al., 2010). Other researchers observed increased P-uptake by wheat and a 30% increase in extractable soil P_i in soils with surface application of RP and compost compared to

applications of compost alone (Verma et al., 2013). In both studies, it was postulated that enhanced rates of N and P mineralization and mobilization occurred when amendments were combined.

The addition of slow-release P fertilizers to soil, such as RP, BM, and composted livestock manure, has unpredictable effects on AMF colonization (Arias et al., 1991; Ryan et al., 1994; Douds et al., 1997; Alloush and Clark, 2001; Gosling et al., 2006). One study found that applications of RP to soil with pH 4.7 increased AMF colonization of corn (*Zea mays* L.) by 35% compared to AMF in soil without RP application (Alloush and Clark, 2001). However another study found that while composted manure increased AMF colonization of corn 8% compared to soil with additions of raw dairy manure, the number of AMF spores in soil did not correspond with the degree of AMF colonization in plant roots (Douds et al., 1997). They concluded that AMF colonization of corn was more influenced by the relative infectivity of AMF spores than compost addition (Douds et al., 1997).

To assess the impact of fertilizer P inputs on soil P availability, it is important to quantify P immobilization and mineralization in soil following amendment application (Tisdale and Nelson, 1975). Mineralization and immobilization of soil P are closely related to the activity of SMB and to microbial biomass P (P_{mic}) (Oberson et al., 1996). Additions of soluble C sources to soil, such as those from compost, increase immobilization of P by P_{mic}, rendering it temporarily unavailable for plants (Buenemann, 2003). The activity of the ALPase enzyme is another indicator of soil P turnover. Activity of ALPase converts P_o to P_i, and originates entirely from microbes (Hebrien and Neal, 1990). Its activity is therefore a direct representation of soil P dynamics as well as soil microbial P activity.

For this experiment, HAP and BM were applied alone and in combination with sheep manure compost (ShMC) to wheat at differing rates in a greenhouse study. Hydroxyapatite was used in lieu of RP due to unavailability of RP. The objectives were to compare effects of pure HAP and BM alone and with ShMC on ALPase activity, P_{mic}, plant P and N uptake, and AMF colonization of wheat. The soils used were from the same site as Chapter 3 and treated organically throughout the experiment.

4.4 Materials and Methods

4.4.1 Experimental design

Soil for the greenhouse study was collected on October 12, 2012 from the Ap horizon (0 to 15 cm) of AAFC-ACS in Scott, SK (Chapter 3). The soil was collected from the organic, LOW diversity level of AAFC-ACS and was cropped with wheat in 2011 and 2012. Soil was collected using a shovel, and several coolers (60 L) were filled with randomly collected, composited soil samples. Upon return to the greenhouse, soil was sieved to 2 mm and air-dried until use.

The experiment was conducted in the U of S greenhouse, which has day and night temperatures of 24 °C/21 °C and a photoperiod of 16 h. One kg field moist soil was weighed into each pot (150 x 180 mm). Coffee filters were placed in the bottom of each pot to allow water drainage but prevent soil loss. The study originally consisted of eight treatments and a control with five replicates of each for a total of 45 pots, but was reduced to 27 pots (three replicates) 30 days after planting (DAP) due to poor seedling emergence.

Treatments consisted of Miracle Gro Organic Choice BM (6-9-0) or HAP applied at the equivalent of 20 kg P ha^{-1} and 40 kg P ha^{-1} (20BM, 40BM, 20HAP, 40HAP) and a combination of BM or HAP applied at the equivalent of 20 kg P ha^{-1} and 40 kg P ha^{-1} with composted sheep

manure (260 g kg^{-1} C, 26 g kg^{-1} N, 3 g kg^{-1} P) applied at the equivalent of 10 tonnes ha^{-1} (20HAP*ShMC, 40HAP*ShMC; 20BM*ShMC, 40BM*ShMC) (Table 4.1). The HAP used for this study was in a powder form.. Although cattle manure is more common in SK than sheep manure, sheep manure was used due to its availability. Furthermore sheep and cattle manure have similar chemical composition (Mubarak et al., 2010) and nutrient availability (Escudero et al., 2012). There were also five replicates of the control pot which had no amendments. The sheep manure compost was air-dried and hand ground to 2 mm with a mortar and pestle. Inorganic C, N, and P content were determined via the digest method of Thomas et al. (1967) described in Section 4.4.6. The compost digestion was performed by technical staff at the U of S. All amendments were incorporated by hand throughout the soil. Application rates were chosen to correspond to previous application rates of RP at this site (Shirtliffe and Knight, 2006).

Ten wheat (cv. Lillian) seeds were planted 2 cm deep in each pot, thinned to four seedlings 14 DAP, and harvested once all plants had flowered (59 DAP). Weed seedlings were removed daily. The experiment was arranged in a completely randomized design, and pots were re-randomized weekly to account for variation from lighting or temperature.

Table 4.1 Analysis and application rates of amendments used in greenhouse study, winter, 2013. HAP=hydroxyapatite, BM=bone meal, and ShMC=sheep manure compost.

	Amendment		
	HAP	BM	ShMC
Carbon (g kg^{-1})	N/A†	350‡	260
Nitrogen (g kg^{-1})	N/A	60	26
Phosphorus (g kg^{-1})	230	90	3.0
	Application Rate		
	HAP	BM	ShMC
Total (mg pot^{-1})	43.5	111	8.65
Nitrogen (mg pot^{-1})	N/A	9.9	0.22
Phosphorus (mg pot^{-1})	10	10	0.026

†Carbon content of BM was not directly measured, but values average 350 g kg^{-1} in the literature (Mondini et al., 2008).
‡HAP was applied alone and in combination with ShMC for both application rates.
§BM was applied alone and in combination with ShMC for both application rates.

4.4.2 Soil characterization

Five soil samples (0 to 15 cm) were collected from AAFC-ACS in Scott, SK in October, 2012 at pre-seeding (PS) with a Dutch auger (5 cm dia x 15 cm depth), composited, transferred field moist to plastic bags and placed in a cooler on site. Soils were stored at 4 °C after returning to the U of S greenhouse. These soils were used for physical and chemical characterization.

Refer to section 3.4.2.1 for a complete description of soil characterization lab protocols. Briefly, 10 g field-moist soil was weighed into an aluminum dish and heated for 24 h at 105 °C to reach a stable oven-dry weight and soil moisture content (SMC) was calculated from Equation 3.1. Inorganic N (NO_3^- and NH_4^+, µg g^{-1}) was extracted with the addition of 50 mL 2 *M* KCl solution to 5.0 g field-moist soil samples. Samples were shaken for 30 min on a rotary shaker (New Brunswick Scientific G10 Gyrotory© Shaker, Edison, NJ) and filtered through #454 filter paper (VWR International LLC, Radnor, PA). Final concentrations of NO_3^- and NH_4^+ were read on the Technicon Autoanalyzer II (Technicon Industrial Systems, Tarrytown, NY, 1973). Plant N uptake was calculated using Equation 3.7.

To extract available phosphate P (μg g^{-1}), 3.00 to 3.09 g dried soil samples were shaken with 30 mL Kelowna solution (28 mL acetic acid, 38.5 g ammonium acetate, 1.11 g ammonium fluoride dissolved in 2 L distilled water) at 160 rpm for 5 min and filtered through #454 filter paper. Final concentrations of available phosphate P were read on the Technicon Autoanalyzer II (Technicon Industrial Systems, Tarrytown, NY, 1973) and plant P uptake was calculated using Eq. 3.7.

Soil pH was determined as outlined by Hendershot et al. (2008). Twenty mL of deionized water was added to 10 g of each sample, stirred intermittently for 30 min, and allowed to settle for 1 h. The pH was read by a pH meter (Fisher Scientific Accumet pH meter, Singapore).

4.4.3 Alkaline phosphatase enzyme activity

Alkaline phosphatase enzyme activity was measured at PS and flowering. Five soil samples (0 to 15 cm) for ALPase were collected at PS with a Dutch auger (5 cm dia). Soil collected at PS was placed field moist in plastic bags and a cooler on site, and all samples were stored at 4 °C until analysis. Soil samples were also collected by hand from each pot at flowering.

Alkaline phosphatase activity of PS soil was measured in triplicate. Refer to section 3.4.2.2 of this thesis for a complete description of methods. Briefly, 4 mL of modified universal buffer (MUB, 3.025 g tris (hydroxymethyl) aminomethane, 2.90 g maleic acid, 3.50 g citric acid, 1.57 g boric acid, 122 mL 1 *M* NaOH mixed and adjusted to 250 mL with deionized water) adjusted to pH 8.5, 0.25 mL toluene, and 1 mL 0.115 *M* *p*-nitrophenyl phosphate (PNP) solution were added to 1 g samples of air-dried, hand-ground (<2 mm) soil. Samples were incubated at 37 °C for 1 h. One mL 0.5 *M* CaCl and 4 mL 0.5 *M* NaOH were added to incubated samples, and filtered through Whatman™ No 1 filter paper (Fisher Scientific, Ottawa, ON). Controls for each

soil involved the addition of 1 mL PNP solution after additions of 0.5 *M* CaCl and 0.5 *M* NaOH. Concentration of P_i in each sample was related to the degradation of PNP to *p*-nitrophenol over the incubation period (1 h). Optical density was read on a spectrophotometer at 420 nm (DU-6 UV-Visible Spectrophotometer, Beckman Instruments, Inc., Fullterton, CA). Activity of ALPase (µg PNP released g^{-1} soil h^{-1}) was determined by referencing a calibration curve of standards containing 0 to 50 µg *p*-nitrophenol mL^{-1} deionized water.

4.4.4 Microbial biomass phosphorus

Microbial biomass P was measured at PS and flowering. Five surface samples of soil (0 to 5 cm) for P_{mic} were taken at PS with a Dutch auger (5 cm dia) and stored field moist in plastic bags until return to the laboratory, then at 4 °C until analysis. Soils at PS were measured in triplicate. Soil was also taken from each pot at flowering.

Refer to section 3.4.2.5 of this thesis for a complete description of P_{mic} analysis. Soil samples were sieved (<2 mm) and incubated at 40 to 50% field capacity (FC) for 1 wk. Field capacity was determined as described in Section 3.4.2.5 and 100% FC was calculated using Eq. 3.2. The value from Eq. 3.2 was multiplied by 0.45 to obtain 45% FC.

For P_{mic} analysis, approximately 10 g of oven-dried equivalent weight of soil were weighed into 50 mL beakers. Each sample was replicated nine times. Three replicates were fumigated and six were unfumigated. Soils for fumigation were fumigated with vapour from approximately 50 mL chloroform in a vacuum-sealed dessicator for 5 min. Following fumigation the dessicator was sealed and left in the dark for 24 h. Unfumigated soils were placed in a sealed dessicator with approximately 50 g soda lime for 24 h before extraction.

For extraction, 200 mL pH 8.5, 0.5 *M* NaOH and 1 mL deionized water were added to the fumigated replicates and three of the unfumigated replicates, and 200 mL pH 8.5, 0.5 *M* NaOH

and 1 mL P_i spiking solution (250 µg L^{-1} KH_2PO_4) was added to the remaining unfumigated replicates to estimate P_i recovery. All replicates were shaken (New Brunswick Scientific G10 Gyrotory© Shaker, Edison, NJ) at 150 rpm for 30 min and filtered through Whatman™ No. 42 filter paper (Fisher Scientific, Ottawa, ON).

Microbial biomass P (µg P g $soil^{-1}$) was determined by the difference in P_i concentrations of fumigated and unfumigated soil samples. To prepare samples for P_i concentration measurements, samples were acidified to pH 1.5 with 6 mL 0.9 *M* H_2SO_4, refrigerated for 30 min, and centrifuged for 10 min at 25,000 x g at 0 °C (Sorvall SE 6+ Centrifuge, Thermo Scientific, Waltham, MA). The supernatant of each sample was transferred to a 50 mL falcon tube (BD Biosciences, San Jose, CA). Four *M* NaOH and 0.5 *M* H_2SO_4 were added to supernatants of each sample to neutralize pH, and 10% *p*-nitrophenol solution (w/v) was added as an indicator. The NaOH and H_2SO_4 solutions were added to samples with Pasteur pipettes (Fisherbrand™ Pasteur Pipets, Fisher Scientific, Ottawa, ON) until the addition of the 10% *p*-nitrophenol solution did not result in a colour change of the samples.

For colour analysis, 8 mL colour developing solution (250 mL 2.5 *M* H_2SO_4, 75 mL 0.03 *M* ammonium molybdate solution, 50 mL 0.30 *M* ascorbate solution, and 25 mL 0.019 *M* antimony potassium tartrate solution brought to 500 mL with deionized water) was added to each sample and optical density read on a spectrophotometer (DU-6 UV-Visible Spectrophotometer, Beckman Instruments, Inc., Fullerton, CA) at 712 nm. Concentrations of P_i were determined by referencing a calibration curve containing 0 to10 µg P_i L^{-1} standards, and P_{mic} was calculated from P_i concentrations using Equations 3.4, 3.5, and 3.6.

4.4.5 Root sampling and analysis

Roots were sampled by hand at wheat harvest. Soil was dug around plant roots in each pot, and roots were gently shaken to remove large particles of soil. Roots were separated from

shoots with scissors, washed with tap water, and stored in a 50% ethanol solution at 4 °C until stained for AMF colonization determination.

Root colonization by AMF was analyzed using techniques outlined in Section 3.4.3. Briefly, roots were cut into 1 cm segments, placed in cassettes (Fisherbrand™ Histosette II™ Tissue and Biopsy Cassettes, Fisher Scientific, Ottawa, ON) and boiled in a 2 *M* KOH solution for approximately 20 min or until roots were clear. To identify AMF structures, roots were submerged in a 5% black ink and vinegar staining solution to dye AMF structures. Percentage root colonization was determined by placing root segments on a Petri dish (100 x 15 mm). Stained AMF structures were counted on a pre-made grid with 100 intersections spaced 0.5 cm apart.

4.4.6 Above-ground plant sampling and analysis

Roots and above-ground biomass of wheat were harvested by hand at flowering. For a complete description of root sampling refer to Section 4.4.5. Aboveground biomass of wheat was separated from roots by cutting with scissors.

All wheat plants were measured for N and P concentrations via the method of Thomas et al. (1967). Refer to section 3.4.5 of this thesis for a complete description of methods. Plant samples of 0.25 to 0.30 g were dried, hand ground with a mortar and pestle, weighed into 75 mL digest tubes, and heated at 360 °C for 30 min with 5 mL 18 *M* H_2SO_4. Hydrogen peroxide (H_2O_2) was added to each sample in 2 mL aliquots and heated at 360 °C for 30 min until clear (approximately 6 heating cycles). Each set of digest tubes (40 total) had two tubes containing 0.03 g glycine used as a check for N recovery and two tubes used as blanks (no plant material). After the final heating cycle, samples were cooled overnight and deionized water was added to

75 mL. Concentrations of N and P were measured on the Technicon Autoanalyzer II (Technicon Industrial Systems, Tarrytown, NY, 1973) and N and P uptake was calculated with Eq. 3.7.

4.4.7 Statistical analysis

All data was tested for normality using the Shapiro-Wilk test (Shapiro and Wilk, 1965). No transformations were needed. Results were analyzed using one-way analysis of varianace (ANOVA) in SPSS (IBM Corporation, Armonk, NY), and treatment was considered significant at $p \leq 0.05$. Means were compared to the control using the Dunnett's Test and considered significant at $\alpha=0.05$. Orthogonal contrasts were used to compare treatments with ShMC and treatments without ShMC. Contrasts were tested at differences of $p \leq 0.05$.

4.5 Results

At 20 days after emergence (DAE), signs of powdery mildew and aphid infestation appeared on some of the seedlings. The powdery mildew worsened over the following weeks, but was gone by 34 DAE. Signs of aphids worsened until harvest. Plants at 20 DAE began showing signs of N-deficiency in the yellowing of leaf tips.

4.5.1 Soil characterization

Soil NO_3-N (3.24 kg ha^{-1}) and PO_4-P (8.61 kg ha^{-1}) levels were deficient (<20 kg ha^{-1}) in this study. The soil pH (5.0) was below 6 and conducive to P solubilization from BM and HAP.

4.5.2 Microbial phosphorus and arbuscular mycorrhizal fungi colonization

Activity of ALPase and colonization of wheat by AMF were lower than the control in 20BM, but otherwise did not differ from the control ($p>0.05$) (Table 4.2, Fig. 4.1, 4.3). Both followed patterns similar to one another, but did not respond consistently to amendment application.

Microbial biomass P did not statistically differ from the control (p>0.05) (Table 4.2, Fig. 4.2). Despite the organic components of BM and ShMC, P_{mic} activity was unaffected by these treatments apart from 20HAP*ShMC which tended to be greater than the control (100.7 µg g^{-1} vs. 36.8 µg g^{-1}). Additions of HAP without ShMC decreased P_{mic} compared to the control. The 20HAP treatment (5.32 µg g^{-1}) had lower P_{mic} than the control, and 40HAP had no P_{mic} (0 µg g^{-1}). Contrast analysis indicated no effect from ShMC compared to treatments without ShMC on AMF, ALPase, or P_{mic} (data not shown).

Table 4.2. Dunnett's test results of alkaline phosphatase (ALPase) activity, microbial biomass phosphorus (P_{mic}), and arbuscular mycorrhizal fungi (AMF) colonization of wheat in organically managed soil amended or not amended (Ct) with 20 or 40 kg P ha^{-1} hydroxyapatite (20HAP, 40HAP), or 20 or 40 kg P ha^{-1} bone meal (20BM, 40BM) alone or in combination with 10 tonnes ha^{-1} sheep manure compost (ShMC) after wheat was grown for 59 days. Pre-seeding (PS) soil was also measured.

	Treatment Number	ALPase	P_{mic}	AMF
PS	0	0†	0	0
Ct	1	0	0	0
20HAP	2	0	-1	0
20BM	3	-1	0	-1
40HAP	4	0	-1	0
40BM	5	0	0	0
20HAP*ShMC	6	0	0	0
20BM*ShMC	7	0	0	0
40HAP*ShMC	8	0	0	0
40BM*ShMC	9	0	0	0

†Negative numbers denote means lower than the control, positive numbers indicate means greater than the control, and 0 indicates no difference from the control.

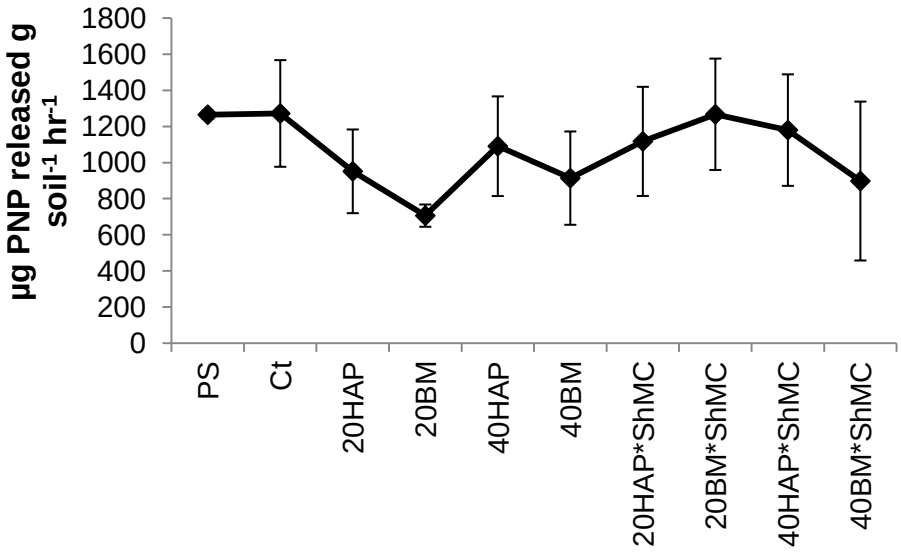

Fig. 4.1 Alkaline phosphatase activity in organically managed soil amended or not amended (Ct) with 20 or 40 kg P ha^{-1} hydroxyapatite (20HAP, 40HAP), or 20 or 40 kg P ha P ha^{-1} bone meal (20BM, 40BM) alone or in combination with 10 tonnes ha^{-1} sheep manure compost (ShMC) after wheat was grown for 59 days. Pre-seeding (PS) soil was also measured. Error bars are one standard deviation.

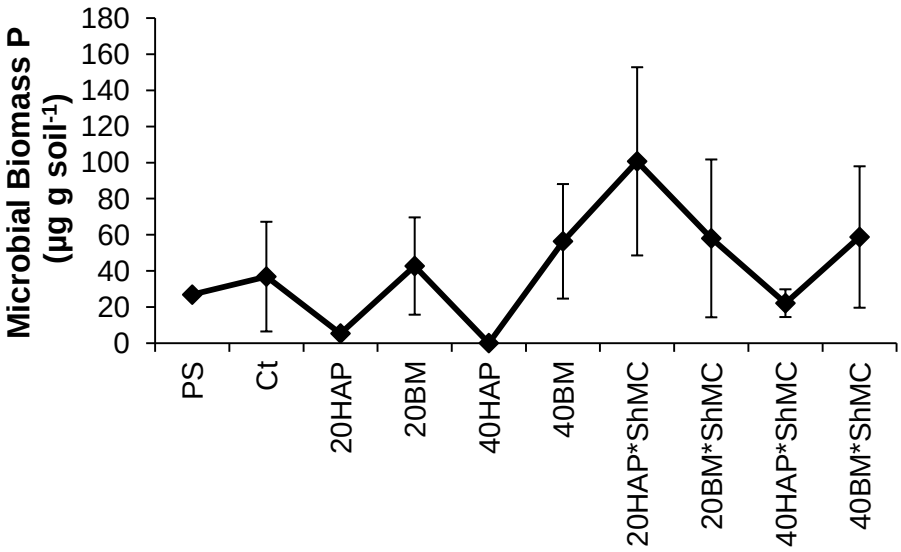

Fig. 4.2. Microbial biomass phosphorus (P) in organically managed soil amended or not amended (Ct) with 20 or 40 kg P ha^{-1} hydroxyapatite (20HAP, 40HAP), or 20 or 40 kg P ha P ha^{-1} bone meal (20BM, 40BM) alone or in combination with 10 tonnes ha^{-1} sheep manure compost (ShMC) after wheat was grown for 59 days. Pre-seeding (PS) soil was also measured. Error bars are one standard deviation.

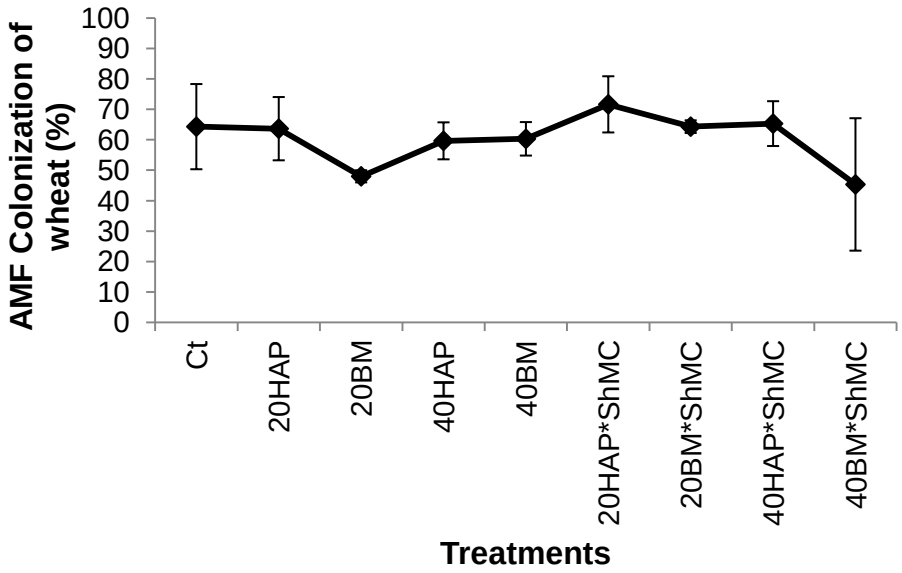

Fig. 4.3. Arbuscular mycorrhizal fungi (AMF) colonization of wheat in organically managed soil amended or not amended (Ct) with 20 or 40 kg P ha^{-1} hydroxyapatite (20HAP, 40HAP), or 20 or 40 kg P ha P ha^{-1} bone meal (20BM, 40BM) alone or in combination with 10 tonnes ha^{-1} sheep manure compost (ShMC) after wheat was grown for 59 days. Error bars are one standard deviation.

4.5.3 Plant phosphorus and nitrogen concentration and uptake

Dry shoot weights differed between treatments (p=0.02) (Table 4.3, Table 4.4). Wheat dry shoot weights of 20HAP*ShMC and 20BM*ShMC were greater (7.63 g pot^{-1}, 8.10 g pot^{-1}) than the control (5.47 g pot^{-1}), and wheat dry shoot weights of the remaining ShMC treatments did not statistically differ from the control. Dry shoot weights of wheat did not differ in any of the remaining treatments where BM or HAP were applied without ShMC apart from lower dry shoot weights in 20BM (3.33 g pot^{-1}).

Wheat P concentrations differed from the control (p=0.001) (Table 4.3, Table 4.5). Wheat had greater concentrations of P than the control in all ShMC treatments (20HAP*ShMC: 2.00 mg g^{-1}, 20BM*ShMC: 2.00 mg g^{-1}, 40HAP*ShMC: 1.90 mg g^{-1}, 40BM*ShMC: 2.07 mg g^{-1}) and

lower P concentrations of wheat than the control in 40BM (1.33 mg g^{-1}). Uptake of P in wheat was higher than the control (0.78 mg pot^{-1}) for the 20HAP*ShMC (1.49 mg pot^{-1}) and 20BM*ShMC (1.61 mg pot^{-1}) and tended to be higher in wheat of the 40HAP*ShMC (1.04 mg pot^{-1}), and 40BM*ShMC (0.91 mg pot^{-1}) treatments and lower than the control for the 40BM treatment (0.622 mg pot^{-1}). Contrast analysis revealed a significant effect of ShMC treatments on P concentrations and uptake of wheat (Table 4.5), but not on dry shoot weights, wheat N concentrations, or wheat N uptake (data not shown). Treatments without ShMC did not have increased plant P concentrations or uptake compared to the control, and wheat N uptake did not differ from the control in any treatment apart from lower N uptake of wheat in the 20BM treatment (2.4 mg pot^{-1}) compared to the control (3.4 mg pot^{-1}). Wheat N concentrations did not differ apart from the 20HAP*ShMC treatment (4.63 mg g^{-1}), which tended to be lower than the control (6.40 mg g^{-1})

Table 4.3. Dunnett's test results of dry shoot weights, phosphorus (P) concentration, P uptake, nitrogen (N) concentration, and N uptake of wheat in organically managed soil amended or not amended (Ct) with 20 or 40 kg P ha^{-1} hydroxyapatite (20HAP, 40HAP), or 20 or 40 kg P ha^{-1} bone meal (20BM, 40BM) alone or in combination with 10 tonnes ha^{-1} sheep manure compost (ShMC) after wheat was grown for 59 days.

	Dry Shoot Weights	Wheat P concentration	Wheat P Uptake	Wheat N Concentration	Wheat N Uptake
Ct	0†	0	0	0	0
20HAP	0	0	0	0	0
20BM	-1	0	0	0	-1
40HAP	0	0	0	0	0
40BM	0	-1	-1	0	0
20HAP*ShMC	+1	+1	+1	0	0
20BM*ShMC	+1	+1	+1	0	0
40HAP*ShMC	0	+1	0	0	0
40BM*ShMC	0	+1	0	0	0

†Negative numbers denote means lower than the control, positive numbers denote means greater than the control, and 0 denotes no difference from the control.

Table 4.4. Dry shoot weights of wheat in organically managed soil amended or not amended (Ct) with 20 or 40 kg P ha^{-1} hydroxyapatite (20HAP, 40HAP), or 20 or 40 kg P ha P ha^{-1} bone meal (20BM, 40BM) alone or in combination with 10 tonnes ha^{-1} sheep manure compost (ShMC) after wheat was grown for 59 days. Treatments different from the control according to Dunnett's test at $p \leq 0.05$ are italicized and in bold.

Treatment	Dry Shoot Weight (g pot^{-1})
Ct	5.47
20HAP	6.30
20BM	***3.33***
40HAP	5.57
40BM	4.67
20HAP*ShMC	***7.63***
20BM*ShMC	***8.10***
40HAP*ShMC	5.40
40BM*ShMC	4.17

Table 4.5. Plant phosphorus (P) and nitrogen (N) concentration and uptake of wheat in organically managed soil amended or not amended (Ct) with 20 or 40 kg P ha^{-1} hydroxyapatite (20HAP, 40HAP), or 20 or 40 kg P ha^{-1} bone meal (20BM, 40BM) alone or in combination with 10 tonnes ha^{-1} sheep manure compost (ShMC) after wheat was grown for 59 days. Treatments different from the control according to Dunnett's test at $p \leq 0.05$ are italicized and in bold.

		Plant P concentration	Plant P uptake
Treatment Number	Treatment	mg g^{-1}	mg pot^{-1}
1	Ct	1.43	0.780
2	20HAP	1.23	0.786
3	20BM	1.60	0.540
4	40HAP	1.50	0.799
5	***40BM***	***1.33***	***0.622***
6	***20HAP*ShMC***	***2.00***	***1.49***
7	***20BM*ShMC***	***2.00***	***1.61***
8	***40HAP*ShMC***	***1.90***	***1.04***
9	***40BM*ShMC***	***2.07***	***0.91***
Treatments Compared	Contrasts	Difference between means	
2,3,4,5-6,7,8,9	20HAP, 20BM, 40HAP, 40BM-20HAP*ShMC, 20BM*ShMC, 40HAP*ShMC, 40BM*ShMC	-0.60**†	-0.60**
		Plant N concentration	Plant N uptake
Treatment Number	Treatment	mg g^{-1}	mg pot^{-1}
1	Ct	6.40	3.4
2	20HAP	6.00	3.9
3	20BM	7.20	***2.4***
4	40HAP	6.40	3.3
5	40BM	6.70	3.1
6	20HAP*ShMC	***4.63***	3.6
7	20BM*ShMC	7.60	3.7
8	40HAP*ShMC	6.90	3.2
9	40BM*ShMC	8.40	3.5

†** indiciates significance at $p \leq 0.05$.

4.6 Discussion

Colonization of wheat by AMF did not follow consistent patterns. It was lower than the control in 20BM and tended to be lower than the control in 40BM*ShMC, but did not differ from the control in any other BM treatments. No other treatments differed from the control. Crop colonization by AMF often does not respond consistently to additions of slow-release P fertilizers to soil such as HAP, BM, and composted animal manures (Pasolon et al., 1993; Douds et al., 1997; Jordan et al., 2000). One study examining composted manure application on AMF colonization of corn found colonization was 49.7% after leaf and manure compost application, 41.5% after raw dairy manure application, and 36.1% after inorganic P fertilizer application, but no correlation was found between the number of AMF spores and AMF colonization levels of corn (Douds et al., 1997). They postulated that the infectivity of AMF species varies, and that some spores may have been dormant (Douds et al., 1997). Therefore colonization of crops is likely more related to the infectivity potential of individual AMF species than slow-release P amendment application. The number of AMF spores and specific species of AMF spores were not measured for this study, but it is possible that different species with different infectivity levels were present in each plot, which resulted in inconsistent AMF colonization response to HAP, BM, and ShMC application.

As seen for AMF colonization of wheat, ALPase activity was lower than the control in 20BM and trended lower in 40BM*CC. The activity of ALPase has often been associated with AMF metabolic activity (Gianinazzi-Pearson and Gianinazzi, 1978; Jabaji-Hare et al., 1990; Tisserant et al., 1993; Guillemin et al., 1995). One study noted that ALPase activity in the roots of mycorrhizal *Allium porrum* and *Platanus acerifolia* jumped 4-45% directly before AMF colonization of each plant (Tisserant et al., 1993). This activity was not seen in roots of the non-

mycorrhizal control (Tisserant et al., 1993). Activity of ALPase originates entirely from microbes (Hebrien and Neal,1990), and staining of roots revealed that AMF mycelium were the source of ALPase activity (Tisserant et al., 1993).

Activity of ALPase and activity of AMF often respond similarly to P fertilization (Guillemin et al., 1995; Balaz and Vosatka, 1997). One study reported that P fertilization decreased both AMF colonization and ALPase activity in roots of *Calamagrostis villosa* compared to soil without P fertilization (Balaz and Vosatka, 1997). Another study found that the proportion of living AMF mycelium with ALPase activity was decreased in soil with P fertilization (0.46) compared to soil without P fertilization (0.97) at six weeks following fertilizer application (Guillemin et al., 1995). While soil rather than roots was measured for ALPase activity in this study, ALPase activity originates entirely from microbial (i.e. fungal) activity (Hebrien and Neal, 1990). Thus ALPase activity from AMF mycelium would also be detected in soil (Hebrien and Neal, 1990). The similar behavior of ALPase and AMF following BM, HAP, and ShMC application likely reflects the linkage between ALPase and AMF rather than amendment application.

Treatments containing ShMC had the greatest P concentrations and uptake of wheat. The P content from manure is preserved during the composting process, and P_i generally comprises 75-90% of total P in compost sources (Eghball et al., 2002). Several studies have found immediate availability of P from composted manure with varying total P concentrations, some very low (<0.01% total P) (Eghball and Power, 1999; Leytem and Westermann, 2005; Sikora and Enkiri, 2005). Uptake of P by fescue (*Lolium arundinaceum*) in an organic system did not differ between soils amended with composted manure (<0.01% total P) and soils amended with triplesuperphosphate (Sikora and Enkiri, 2005). A further study noted that additions of

composted manure (0.3% total P) to organically-managed soils saw P-uptake of corn that was equal to or greater than P-uptake of corn in conventionally-managed soils amended with ammonium nitrate (Eghball and Power, 1999). These studies suggest that P from composted manure is immediately available across a wide range of total P concentrations. Although the effects of ShMC alone were not tested, it is likely that inorganic P was readily available from ShMC application in this experiment.

The C:N ratio of ShMC (10) is less than 20, which implies that net mineralization of N will occur (Tisdale and Nelson, 1975). However N concentration and uptake of wheat in all ShMC treatments were unaffected apart from the lower N concentration in 20HAP*ShMC. The low N concentration in wheat of 20HAP*ShMC was attributed to the dry shoot weights of this treatment, which were greater than most treatments and resulted in dilution of N in the plant tissue.

The lack of difference in N uptake and concentrations of wheat in ShMC treatments was likely due to the conversion of N to organic forms during the composting process. Up to 50% of N in manure is converted to organic forms during composting, resulting in 0% to 30% N availability from compost in the first year of its application (Sikora and Szmidt, 2001). The loss of N results in narrowing of the N:P ratio, which induces mineralization of P (Adler and Sikora, 2003). One study in SK found that N from composted manure showed greatest plant N uptake and concentrations in the second year after application, as organic forms of N mineralized (Brandt et al., 2007). It is likely that if this experiment had been conducted over multiple years, more N would become available over time.

Dry shoot weights were greatest in the 20HAP*ShMC and 20BM*ShMC treatments. The addition of compost to soil can increase shoot weights and accelerate maturity of plants (Perner

et al., 2006; Jahromi et al., 2012). Applications of 40% compost (v/v) versus 20% compost (v/v) increased dry shoot weights (5.3 to 7.2 g pot^{-1}) of leek (*Allium ampeloprasum* L.) in a greenhouse study (Perner et al., 2006). Another study noted that dry shoot weights of tomato (*Lycopersicum esculentum* Mill.) and cucumber (*Cucumis sativus* L.) increased with compost additions (Jahromi et al., 2012). The benefits from compost to dry shoot weights are largely due to an increased supply of P and N, which is necessary for plant growth (Tisdale and Nelson, 1975). Phosphorus in particular is necessary for the growth of reproductive organs and early crop development (Tisdale and Nelson, 1975).

Dry shoot weights of wheat in 40HAP*ShMC and 40BM*ShMC were not greater than the control, although P concentrations and uptake were. The over application of P does not result in decreased crop yield (Nelson and Janke, 2007), therefore the smaller dry shoots of wheat in these treatments are unclear. This may be a result of damage to wheat from aphids.

It is also possible that the combination of HAP and BM with ShMC increased P-availability from HAP and BM. Previous studies have shown increased crop yield and plant nutrient uptake when BM and RP (HAP) are combined with compost than when applied alone (Nishanth and Biswas, 2008; González et al., 2010; Verma et al., 2013). One study found that applications of vermicompost and BM together to soil with pH 6.9 showed greater beet (*Beta vulgaris*) yield (3.85 kg m^2 vs. 3.77 kg m^2) and greater N and P plant concentrations (24.20 and 4.50 mg kg^{-1} vs. 23.48 and 4.12 mg kg^{-1}) than vermicompost applied alone (González et al., 2010). They postulated that the combination of amendments induced more rapid N and P mineralization. A further study found that surface applications of compost combined with RP in a mid-pH soil (7.7) increased P-uptake of wheat and extractable soil P_i by 30% compared to all other treatments (Verma et al., 2013). They attributed this increase in P availability to the

mobilization of P from RP, as treatments with compost alone did not show these increases (Verma et al., 2013). The soil pH of this study (5.0) is more conducive to BM and HAP dissolution than the aforementioned studies, so it is possible that the combination of HAP and BM with ShMC increased P mobilization compared to BM and HAP applied alone.

Overall, treatments without ShMC were very ineffective for increasing plant P and N concentration and uptake. Bone meal in particular was very ineffective, as evidenced by the low dry shoot weights and N uptake of wheat in 20BM and the low P concentrations and uptake of wheat in 40BM. The pH of the greenhouse soil (5.0) is below 6.0 and conducive to P-solubilization from HAP and BM (Bekele and Hofner, 1993; Surendra et al., 1993), but the 20HAP, 40HAP, 20BM, and 40BM treatments did not provide greater P or N for wheat than the control treatment. This is likely due to low solubility of the material. It is also possible that the application rates of HAP (20 kg P ha^{-1} and 40 kg P ha^{-1}) were too low to see any yield response from wheat. One study found that RP applied at 40 kg ha^{-1} did not increase yield of wheat in soil with pH 5.5 compared to soil with no applications of RP (Dann et al., 1996). In fact several studies have seen no increase of wheat yields following RP application unless RP is applied at 65 kg ha^{-1} or higher (Adediran et al., 1998; Zaharah and Bah, 1997; Gatiboni et al., 2003).

The activity of P_{mic} was largely unaffected among ShMC treatments. Apart from 20HAP*ShMC, treatments with ShMC did not vary from the control. Normally P_{mic} activity is closely related to the amount of soluble C added to soil (Buenemann, 2003; Ofori-Frimpong and Rowell, 1999), which can mineralize following additions of compost (Mäder et al., 2002; Fliessbach et al, 2007). However mineralization of C from ShMC did not contribute to P_{mic} aside from 20HAP*ShMC.

The decomposition of composted manures in soil is partially related to the concentration of low molecular weight (i.e. sugars, proteins, water soluble C) and high molecular weight (i.e. lignins, polyphenols) components in the organic matter (Mubarak et al., 2010). One study found that farmyard manure (including sheep) contained twice as many polyphenols and approximately 100 more g kg^{-1} lignins than chicken manure (Mubarak et al., 2010). The polyphenol and lignin content of ShMC was not measured, but it is possible the composition of ShMC retarded mineralization of C, which in turn did not provide a soluble C source for P_{mic}. However this does not explain the inconsistent response of P_{mic} to ShMC. Wheat in 20HAP*ShMC has the highest P_{mic} of all treatments, while 40HAP*ShMC is much lower.

The P_{mic} of all treatments containing BM also did not differ from the control. Despite previous findings to the contrary, the organic component of BM did not provide C substrate for P_{mic} (Novelo et al., 1998; Jeng et al., 2004 and 2006; Mondini et al., 2008). One study reported that application of BM at 400 kg N ha^{-1} resulted in a significant (50%) increase in SMB size after 2 d compared to no application of BM in both acidic and alkaline soils (Mondini et al., 2008). The lack of a difference in P_{mic} following BM application in this study suggests that very little C dissolved from BM for use as microbial substrate. It may also suggest that P_{mic} would respond to greater application rates of BM to soil than were used in this experiment. Results may also suggest that the C component of BM may have been in the form of carbonate, as is common in bone mineral (Rey et al., 2009). This is an inorganic form of C which would not provide substrate for P_{mic} (Schärer, 2003; Oberson and Joner, 2005).

Application of HAP negatively impacted P_{mic} size. Hydroxyapatite does not contain C, and was not expected to influence P_{mic} due to lack of C mineralization. While P_{mic} in 20HAP*ShMC is the greatest of all treatments, P_{mic} in 40HAP*ShMC is much lower in

comparison. This suggests that the negative impact of HAP is not apparent until the higher application rate. This is also seen in the 20HAP and 40HAP treatments. Microbial biomass P in 20HAP is lower than the control, and in 40HAP, it is absent. The detrimental effects to P_{mic} from HAP indicate that not only is it not a source of C, but it has components that are actively harming P_{mic}. It is possible that some by-product that is toxic to P_{mic} contaminated the reagent.

Overall, the application of BM, HAP, and ShMC did not have consistent effects on AMF colonization or ALPase activity. Rather than the effects from individual organic fertilizers on both of these processes, it is likely that colonization of wheat by AMF is more influenced by the individual species of AMF present in soil and their infectivity potential rather than application of amendments. Similarly, the activity of ALPase indicates it is linked to AMF activity rather than amendment addition.

Treatments without ShMC did not contribute to wheat P uptake or concentrations. Despite soil pH (5) conducive to P solubilization from BM and HAP, these amendments did not release P in soil for uptake by wheat. This may also be due to the low application rates of this study. It is also possible that combinations of BM with ShMC and HAP with ShMC increased P solubilization from both. Unlike P, uptake and concentrations of N in wheat were not affected by any treatment. Nitrogen becomes less available as a result of the composting process and is generally far less available than P in the first year of composted manure application.

Applications of ShMC and BM did not result in greater P_{mic} size and activity despite their organic components, which indicates no or very low mineralization of C from each. This may also indicate that the composition of ShMC was resistant to microbial decomposition. Applications of HAP decreased P_{mic}, which may be a result of toxic impurities in the material.

This experiment shows that composted manure is a valuable resource in organic systems for immediate plant P nutrition. Nitrogen is less available from compost in the short-term, but previous studies have indicated it becomes more available in the second year of application. Thus application of manure to meet N demand of crops can result in soil P loading. Composted manure is therefore a valuable resource for organic farmers, but care must be taken when applying it to meet plant N versus P requirements.

Bone meal and HAP are likely not viable options for organic farmers, although the application rates of this study may have been too low to be effective. Even in soil pH conducive to P solubilization from BM and HAP, they provided no P for ultilization by wheat. Their use as organic P fertilizers in SK is not encouraged, as the soil pH of cultivated soil in the province is generally greater than the soil used in this study (Malhi et al., 2002).

5. SYNTHESIS AND CONCLUSIONS

The purpose of this study was to research methods for organic farmers in SK to increase soil P availability to crops. Throughout the province the immobilization of soil P by the soil microbial biomass and various cations renders it largely unavailable to plants (Malhi et al., 2002). This creates problems in organic farming systems, as the addition of synthetic P fertilizers is prohibited (Canadian General Standards Board, 2011). Thus organic farmers mainly rely on crop rotation, soil amendments like rock phosphate and compost, and soil organisms like arbuscular mycorrhizal fungi to maintain soil P fertility.

The two studies of this thesis centered around one question: What are practical ways for organic farmers in SK to address shortages of inorganic soil P? Although the studies were conducted separately, they are closely related. The first study was conducted in the field and measured the effects of organically-managed crop rotations on AMF colonization, crop P uptake and concentrations, and soil P, and the second study measured the effects of organic fertilizer addition (BM, HAP, and composted sheep manure) on AMF colonization of organically grown wheat, P uptake and concentrations of organically grown wheat (*Triticum aestivum* L.), and soil

P. Plant N uptake and concentrations were also measured in each study, as availability of N is closely related to availability of P.

In the field study, the inclusion of a non-mycorrhizal crop (mustard) and partial fallow (lentil green manure) did not decrease the number of AMF spores or delay AMF colonization compared to cropping sequences with continuous AMF colonization, which is contrary to many previous findings in conventional systems (Gavito and Miller, 1998; Karasawa et al., 2002; Bedini et al., 2007). Futhermore the sequence (wheat-barley) with the lowest levels of soil N also had the lowest AMF colonization of all crops in August (37%). Rather than a crop's place in rotation or root structure, AMF colonization may have been influenced by a combination of N deposition from legumes, deficient (<20 kg P ha^{-1}) levels of soil P, and the presence of host species due to mycorrhizal weeds (Feldmann and Boyle, 1999; Chen et al., 2005). All three conditions are specific to organic systems where synthetic P fertilizers and the use of herbicides are banned. Additionally, P uptake and concentrations of all crops were average at flowering despite the P-deficient soil, which may indicate the importance of AMF and legumes in organic systems.

In the greenhouse study, AMF colonization was unaffected by additions of composted manure, and P concentrations (1.99 mg g^{-1}) and uptake (1.26 mg pot^{-1}) of wheat were greatest in compost treatments on average compared to the control (1.43 mg g^{-1}, 0.71 mg pot^{-1}). These results imply that organic farmers may use compost to increase P uptake of crops without decreasing effectiveness of AMF.

Overall, the experiments showed the importance of crop rotation and composted manure use in organic farming systems. The inclusion of legumes in crop rotation add P and N to soil through their decomposition, and may also preserve AMF communities in organic systems.

Additionally, the use of composted manure may provide a ready source of P without negatively impacting AMF. Thus the combined use of legumes and composted manure may add P to soil directly and indirectly: directly through soil P deposition and amendment application, and indirectly through the preservation of AMF.

In the end, one word stands out from the research question: "practical". In practicality, the small size of herds and large size of organic farms in SK will limit the use of composted manure in the immediate future (Knight et al., 2010a). This means that organic farmers in SK would benefit most from the proper inclusion of legumes in crop rotation, and the use of legume green manures to supply N and P for following crops. It is also important to avoid three-year successions of cereal and/or oilseed crops, as seen in the M-W-B rotation from this study. This sequence was by far the most depleted in available N and P, and also had the lowest AMF colonization in August. The proper use of crop rotation will also help to preserve AMF communities and offset some level of P deficiency. It is unlikely, however, that AMF will increase yields on its own. The most effective organic system utilizes both crop rotation and AMF to maintain crop yield and soil P levels.

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